THE VITAMIN A REQUIREMENT FOR GROWTH OF FOXES AND MINKS AS INFLUENCED BY ASCORBIC ACID AND POTATOES

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Smith ('42) first described the symptoms of vitamin A deficiency in silver foxes. From limited tests he observed that 15 to 25 I.U. of vitamin A per kilogram of body weight daily were necessary to prevent deficiency. There was no appreciable storage, in the liver, of vitamin A until approximately 4 times this amount was fed. Bassett et al. ('46a) extended these studies to determine the vitamin A requirement under actual ranch conditions. They occasionally observed deficiency symptoms on intakes as high as 100 I.U. of vitamin A per kilogram of body weight daily when these pups were drastically depleted of vitamin A before they were weaned. In these experiments the diets were extremely low in carotene or vitamin A and contained some ingredients not commonly used in feeding foxes. It seemed desirable to determine whether or not actual deficiency would be experienced when more conventional rations were fed. As no previous work had been carried on to determine the vitamin A requirements of mink, it was decided to conduct the same type of experiment with that species also.

Mathiesen ('42) reported that foxes do not require a dietary source of ascorbic acid. Since most fur farmers feed vegetables which contain ascorbic acid, however, it seemed important to test its value in the diet.

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EXPERIMENTAL

Eighty silver fox pups and 60 mink kits, divided equally according to sex, were allotted by chance into 10 groups. The foxes were 50 and the minks 64 days of age when started on experiment. The percentage composition of the basal diet was as follows: ground horsemeat 10.0, soybean oil meal 3.5, beef meal 2.9, skim milk powder 0.6, rolled oats 5.8, bread crumbs 5.6, cookie crumbs 11.2, brewers' dried yeast 4.7, wheat germ meal 5.8, ground limestone 1.3, and water 48.6. This diet contained on analysis: protein 28.8%, ether extract 6.4%, crude fiber 2.5%, nitrogen free extract 56.4%, and ash 5.9% on a dry matter basis, and only traces of carotene. The basal diet was supplemented with vitamin A for different groups of animals at the rates of 0, 50, 500, 2500 and 5000 I.U. of vitamin A per pound of moist, mixed feed. Five other groups receiving the above amounts of vitamin A were given, in addition, 20 mg of ascorbic acid per pound of feed to test whether (1) this vitamin would influence the vitamin A requirement of foxes and minks, and (2) vitamin C would affect their growth rates. The foxes were fed once daily, the minks twice, and records were kept of the feed consumed. All animals were weighed at the start of the experiment, and the foxes were weighed each 2 weeks, and the minks each week thereafter. An average of 2 weights taken on successive days constituted the initial and final weights. Weekly observations were made to detect the onset of any deficiency symptoms. Animals were killed when their pelts were prime, and observations made on fur growth and quality and on the general condition of the animals.

Blood samples obtained by cardiac puncture were cooled and shipped by parcel post in the late afternoon following collection to Cornell University, Ithaca, N. Y. Immediately after their arrival the following day, the samples were centrifuged and analyzed for carotene and vitamin A. It is possible that slight losses of vitamin A occurred from this handling, although tests have shown that vitamin A is not appreciably reduced when plasma or even whole blood is stored at approx-

imately 35°F. for at least a week (Loosli, '47). Livers collected from all minks and from at least 5 foxes in each lot were ground and a composite sample frozen and stored 6 to 7 weeks before vitamin A analysis was made.

Comparative pelt quality was determined by experts of a New York fur auction company.

RESULTS

Studies with foxes

There were no appreciable differences in rates of growth on the various intakes of vitamin A or ascorbic acid. Total food intake appeared to be slightly greater when the diet contained 500 I.U. or more of vitamin A per pound, but no ascorbic acid, than lower levels. The quality of the pelts of foxes appeared to be slightly less desirable on low intakes of vitamin A unless ascorbic acid was fed, but the number of animals studied was too small to show a significant trend.

Symptoms of vitamin A deficiency (i.e., head cocking, whirling, and weaving or staggering gait) were seen in 38% of the foxes receiving no extra vitamin A or ascorbic acid, while the addition of 20 mg of ascorbic acid per pound of feed prevented the occurrence of symptoms.

That there may be an interrelationship between vitamin A and ascorbic acid in the nutrition of foxes is further supported by the vitamin A analyses of blood serum and livers (table 1).

There appears to be a higher level of vitamin A in the blood serum and livers on the vitamin-A free diets with ascorbic acid than on the diet containing neither of these vitamins. There was a consistent increase in the vitamin A content of the blood serum and livers of foxes as the dietary intake rose.

Studies with minks

With the possible exception of the 0 vitamin A level, there were no differences in gain in weight which can be correlated with the amount of vitamin A or with the omission of ascorbic acid in the diet. At the 0 vitamin A level the average gain in

weight was 0.20 pound less than the average of all other levels tested, and 0.38 pound less than the value for normal minks of the same strain. This difference may have been due to the low vitamin A intake. Differences in food consumption did not appear to be related to the intakes of vitamin A or ascorbic acid. Vitamin A deficiency symptoms, such as occur in foxes and other animals, were not observed. This study suggests that minks require less vitamin A per pound of feed

TABLE 1

The vitamin A content of the serum and livers of foxes and minks as affected by amount of the vitamin in the feed.

ASCORBIC ACID OF FEED	VITAMIN A PER POUND OF FEED (I.U.)				
	0	50	500	2500	5000
mg/lb.					
	Vitamin A content of blood serum of foxes (μg/ml)				
0	0.28 ± .01 [1]	0.43 ± .03	1.11 ± .06	3.35 ± .37	5.66 ± .82
20	0.63 ± .16	0.39 ± .03	1.30 ± .28	2.21 ± .19	5.40 ± .22
	Vitamin A content of fox livers (μg/gm) [2]				
0	0.15	0.25	0.55	2.86	3.73
20	0.21	0.30	0.62	1.34	3.77
	Vitamin A content of blood serum of minks (μg/ml)				
0	0.22 ± .01	0.19 ± .02	0.23 ± .04	0.78 ± .02	2.64 ± .75
20	0.31 ± .14	0.46 ± .12	0.33 ± .18	1.02 ± .16	1.46 ± .12
	Vitamin A content of mink livers (μg/gm) [2]				
0	0.00	2.06	2.06	18.45	72.10
20	2.06	2.57	2.64	14.60	202.00

[1] Mean and standard error of the mean.

[2] Analyses of composite liver samples.

than foxes, or that their liver reserves at the start of the study (64 days of age) were adequate to carry the control groups until pelting. Death losses among animals that did not receive ascorbic acid were 50, 17, 33, 0 and 33%, respectively, for the 5 levels of vitamin A, in ascending order. Corresponding figures for the animals that were given ascorbic acid were 50, 0, 33, 67 and 17%. Post-mortem examination revealed hemorrhagic areas in the region of the heart and lungs, over

the skull, and throughout the abdomen and intestines. The stomach and intestines frequently contained blood, and in some instances the bladder was enlarged and filled with bloody urine. Emaciation and a severe anemia, indicated by the pale color of the liver, spleen, kidneys, heart and blood, coupled with a blue and transparent appearance of the intestines, were common. A similar pathology has been described by Kennedy ('46).

Examination of the pelts revealed no certain trend in quality related to the intake of vitamin A.

The analyses of blood serum and livers for vitamin A (table 1) show a generally consistent increase in the vitamin A content as the intake of the vitamin rose. In most cases there was also a higher content of vitamin A when ascorbic acid was fed; this was observed also with foxes.

Effects of feeding potatoes to foxes and minks

Potatoes as 50% of the diet were fed to 42 foxes and 48 minks. The percentage composition of the diet was ground meat 10.0, soybean oil meal 3.0, beef meal 2.5, dried skim milk 0.5, oatmeal 5.0, dried brewers' yeast 3.9, irradiated yeast 0.1, wheat germ meal 5.0, cooked potatoes 50.0, and water 20.0. On analysis, the dry matter was found to contain 33.4% protein, 3.7% ether extract, 3.6% crude fiber, 53.8% nitrogen free extract, and 5.5% ash. Half of each group were given a supplement of 5000 I.U. of vitamin A per pound of mixed feed. Data collected are shown in table 2.

DISCUSSION

Foxes that received 5000 I.U. of vitamin A per pound of feed gained an average of 1.05 pounds more than those receiving no supplement. Conversely, minks that received no vitamin A supplement gained an average of 0.18 pound more than those furnished the extra vitamin, largely because of a greater accumulation of fat. Of the minks that were not given additional vitamin A, 79% were fat or extra fat, but only 55%

of those fed the vitamin supplement were considered fat. Some foxes fed the 50% potato diets consumed as much as 2.0 pounds of feed a day. Ordinarily a growing fox does not consume more than 1.25 pounds of feed per day. Those that received 5000 I.U. of vitamin A per pound of feed consumed 35%, and those furnished no supplement 21%, more food than an average of 80 foxes fed a diet that did not include potatoes. Mortality was greater for both species in groups that did not receive extra vitamin A. The content of vitamin A in the blood serum and livers of both foxes and minks fed

TABLE 2

The mortality rate, gain in weight, food intake and vitamin A content of serum and livers of foxes and minks receiving diets containing 50% potatoes with and without added vitamin A.

VITAMIN A PER POUND OF FEED	MORTALITY	AVERAGE GAIN IN WEIGHT	AVERAGE FOOD INTAKE	VITAMIN A CONTENT	
				Serum	Liver
I.U.	%	*lbs.*	*lbs.*	*μg/ml*	*μg/gm*
			Foxes		
0	14	8.58	239.3	0.92 ± .09	0.45
5000	0	9.63	279.0	6.45 ± .52	6.07
			Minks		
0	21	1.35	79.8	0.32 ± .08	23.40
5000	8	1.17	76.7	3.64 ± .28	363.00

potatoes was higher than for comparable animals not fed potatoes. Because vitamin A was added per unit of diet, the intake increased with the greater food consumption. It is not clear whether this would fully explain the higher serum and liver contents, or whether the potatoes may have enhanced the utilization or storage of the vitamin. The influence of ascorbic acid and of potatoes in increasing supplies of vitamin A in the serum and liver is clearly shown in table 3. The effect here observed appears to be similar to the sparing action of ascorbic acid when fed with low intakes of carotene (Hickman et al., '44). A relationship between vitamins A and C in calf nutrition was reported by Boyer et al. ('42).

The magnitude of some of the increased stores of vitamin A and the influence, for all species, of antioxidants and other dietary ingredients on the conservation and utilization of this vitamin emphasize the need for extension of these studies.

TABLE 3

The influence of ascorbic acid and potatoes on the vitamin A content of the serum and liver.

SUPPLEMENT	AVERAGE VITAMIN A CONTENT WHEN —			
	No vitamin A fed		5000 I.U. vitamin A fed per pound of diet	
	Serum	Liver	Serum	Liver
	μg/ml	μg/gm	μg/ml	μg/gm
		Foxes		
Basal only	0.28	0.15	5.66	3.73
Basal + ascorbic acid	0.63	0.21	6.40	3.77
Basal + potatoes	0.92	0.45	6.45	6.07
		Minks		
Basal only	0.22	0.00	2.64	72.10
Basal + ascorbic acid	0.31	2.06	1.46	202.00
Basal + potatoes	0.32	23.40	3.64	363.00

TABLE 4

Influence of the vitamin A intake upon the serum and liver content of growing foxes.

VIT. A INTAKE/ KG BODY WT.	NO. OF FOXES	AVERAGE VIT. A CONTENT		VIT. A INTAKE/ KG BODY WT.	NO. OF FOXES	AVERAGE VIT. A CONTENT	
		Blood serum	Liver			Blood serum	Liver
I.U.		μg/ml	μg/gm	*I.U.*		μg/ml	μg/gm
0	30	0.00	0.00	200	6	1.98	1.20
15	19	0.00	0.00	600	6	3.43	1.61
20	10	0.00	0.00	830	16	2.78	2.10
25	24	0.32	0.25	1825	16	6.03	3.75
50	24	0.40	0.26	2500 [1]	21	6.45	6.07
100	16	0.55	0.40	5000	3	0.81	6.13

[1] Diet contained 50% potatoes.

The available data for foxes (Smith, '42; Bassett et al., '43, '46a) showing the vitamin A intake and corresponding blood and liver contents are summarized in table 4. These data

show that a vitamin A intake of 25 I.U. per kilo of body weight gave measurable amounts in the blood and liver, whereas these tissues were practically devoid of vitamin A on an intake of 20 I.U. Moore et al. ('43) found that in calves 15 μg of vitamin A per 100 ml plasma are necessary to prevent deficiency symptoms. Thus, using this criterion, 25 I.U. per kilo of body weight (11.4 I.U. per pound of weight, or 2.8 μg), which is approximately equivalent to 10 I.U. per pound of moist diet, may be considered the minimum requirement for the growth of foxes. This minimum requirement (6.2 μg — assuming 1 μg = 4 I.U.) is the same as that found for rats and farm animals by Guilbert et al. ('40), and supports their hypothesis that the vitamin A requirement is related to body weight and that it is approximately the same for all mammals. Bassett et al. ('46b) have also shown that foxes utilize carotene as a source of vitamin A. The quantitative requirement, while not yet accurately defined, is not out of line with the predicted need based on studies with other animals.

A higher apparent requirement of vitamin A to prevent the occurrence of deficiency symptoms reported by Bassett et al. ('46a) appears to have been the result of severe depletion of the pups before they were started on the diets containing the vitamin A supplements.

The present study further emphasizes the view that both "growing" and "pelting" diets fed to young foxes and minks, which usually include vegetables and fresh liver, contain sufficient vitamin A, and therefore the addition of this vitamin or of carotene concentrates is unnecessary.

SUMMARY AND CONCLUSIONS

Eighty silver foxes and 60 mink kits were allotted to experimental groups receiving a basal diet low in vitamin A and supplemented with 0, 50, 500, 2500 and 5000 I.U. of vitamin A per pound of feed. Half of each of the above groups received 20 mg of ascorbic acid per pound of feed. Blood samples, taken by cardiac puncture, and livers were collected and assayed for vitamin A.

Gross vitamin deficiency symptoms appeared among foxes on the unsupplemented basal ration, but diets containing 50 I.U. or more of added vitamin A per pound were satisfactory for growth, prevented deficiency symptoms, and provided a vitamin A content in the blood and liver that rose consistently as the intake increased. The addition to the basal diet of 20 mg of ascorbic acid per pound of feed apparently prevented the appearance of vitamin A deficiency symptoms and increased slightly the vitamin A content of the blood serum and liver.

The content of vitamin A in the blood serum and livers of minks also increased consistently with the intake of the vitamin, and was greater when ascorbic acid was added to the basal diet. Other differences could not be correlated with either vitamin A or ascorbic acid.

Results indicate that an interrelationship exists between vitamin A and ascorbic acid in the nutrition of foxes and minks.

A diet containing 50% cooked potatoes was fed to 42 silver foxes and 48 minks. Food intakes and the content of vitamin A in the blood serum and liver were higher than for comparable animals not fed potatoes.

The minimum vitamin A requirement necessary to provide satisfactory growth and a detectable content of vitamin A in the blood serum and liver of foxes is approximately 25 I.U. per kilogram of body weight per day. This is equivalent to 6.2 μg per kilo (11.4 I.U., or 2.8 μg per pound of body weight), which can be supplied by adding approximately 10 I.U. of vitamin A per pound of the moist diet.

Under ordinary ranch conditions where little or no vitamin A depletion occurs among fox pups during the suckling period, diets of the type fed by most ranchers contain sufficient vitamin A so that no supplementation is necessary. The inclusion of adequate amounts of leafy vegetables, tomatoes, apples, or a substantial quantity of cooked potatoes in the diet would preclude the necessity for vitamin A supplementation.

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COMPARATIVE GROWTH-PROMOTING VALUES OF THE PROTEINS OF CEREAL GRAINS

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Close to one-third of the total protein in the human dietary in the United States is supplied by cereals, chiefly wheat, corn, oats, rye, barley and rice. Cereal grains have been from antiquity an important source of protein in the human diet.

Numerous studies have been conducted by feeding experiments over a period of many years on the comparative nutritive value of the proteins of cereal grains. Recorded results of much of the earlier work, however, are confusing and conflicting. Not infrequently it was concluded that the grains differed little, if any, among each other, with respect to their protein nutritional properties, and that they were all of rather low value. These investigations were conducted in different laboratories, under various conditions, and in most cases on only a few of the grains at the same time. Because of lack of uniformity in experimental procedures, variations in the compositions of basal diets and inadequacy of vitamin and mineral supplements, the results do not afford a satisfactory basis for comparing the cereal grains with respect to the nutritive value of their proteins.

Several of the early investigators on the protein nutritional value of cereal grains concluded that rye, wheat, corn, barley and oats vary little with respect to the nutritive value of their proteins (Sherman and Winters, '18; McCollum et al., '19;

Osborne and Mendel, '20; McCollum and Simmonds, '25; Johnson and Palmer, '34).

Recorded data on the nutritive value of barley protein are few, and the work was done many years ago. The values observed are conflicting and they do not afford a satisfactory basis for comparison with those of other grains. Steenbock et al. ('18) and McCollum et al. ('19) found comparatively low values for barley protein. Working with the entire ground seed, Osborne and Mendel ('20) stated that the results "leave no doubt of the adequacy of the barley proteins as a whole in the nutrition of growth." With pearled barley, however, the animals (rats) grew very slowly.

Corn has been generally regarded as an inadequate source of protein for growing animals. Zein, constituting from 41 to 52% of the protein of corn, is well-known to contain little or no tryptophane and to be low in lysine, and these amino acids are not satisfactorily compensated by the other proteins present. Maynard, Fronda and Chen ('23) found corn to have a low protein efficiency. Grau ('46) showed that corn gluten meal had a low growth value for chicks and that it required addition of arginine, lysine and tryptophane. Boas-Fixsen et al. ('34), in nitrogen equilibrium and growth studies, concluded that only a slight difference exists between the nutritive value of the proteins of whole wheat and whole maize for support of growth in the young rat.

Compared with most cereal grains, rye has received little attention with regard to its protein value. Osborne and Mendel ('20) reported that the efficiency of rye protein is comparable with that of other grains studied. Later, however, in re-evaluating Osborne and Mendel's data, Mitchell and Hamilton ('29) stated that the superiority of rye protein over wheat is clearly demonstrated. McCollum et al. ('19) observed that rats grew at only approximately half the normal rate. In feeding experiments with rye and wheat breads, Kon and Markuze ('31) obtained results indicating the superiority of the rye breads with respect to the biological value of the protein. Johnson and Palmer ('34) concluded that rye protein

is comparable with that of wheat, corn and oats, and that lysine, but not tryptophane, is the first limiting factor.

Considerable confusion exists in the earlier publications regarding the nutritive value of the protein of oats. Conflicting statements may be found by the same authors at different times, as pointed out by Osborne and Mendel ('20). In general, however, the conclusions point to a higher value for the protein of oats than for most of the other cereal proteins. In nitrogen balance studies with human adults, Sherman et al. ('19) found that the protein of oats and maize had about equal value. By means of nitrogen metabolism experiments with rats, Mitchell ('24) showed a distinct superiority of oat protein over corn protein at different levels of protein intake. Hoagland and Snider ('27) noted a close similarity between the protein of wheat and oatmeal. Hartwell ('26) found that the protein of oats was of good nutritive value, although the diet was supplemented only with mineral salts and butter. Mitchell and Smuts ('32) showed that addition of lysine to an oat diet resulted in a distinct though slight increase in the growth of rats. The protein of rolled oats was found by Mitchell and Block ('46) definitely superior to that of other cereals tested, whether processed or unprocessed.

That rice does not show the marked differences which other cereal proteins do in respect to their amino acid composition was demonstrated by Osborne et al. ('15). They pointed out that rice protein yields a relatively large amount of each of the basic amino acids, arginine, histidine and lysine. Later, Osborne and Mendel ('18) emphasized the superior growth-promoting value of the protein of rice compared with that of corn. McCollum and Simmonds ('17) found rice protein comparable with that of wheat and corn. In metabolism experiments with rats, Mitchell ('24) demonstrated that rice protein had a higher biological value than that of corn and oats. Li ('30) found rice superior to barley. Recently, Sure ('46, '47) demonstrated a marked superiority in growth-promoting value and in protein efficiency of polished rice over that of wheat flour. Mitchell and Block ('46) reported

that lysine is the limiting amino acid in rice protein. In feeding experiments with rats, Kik ('39) found that polished rice and brown rice have essentially the same growth value, but that brown rice protein is better utilized, having a biological value of 72.7 compared with 66.6 for polished rice. Kik ('40) also found that cystine, methionine and lysine supplement the proteins of whole and of polished rice to a slight extent. Addition of tryptophane did not confer any beneficial effect.

EXPERIMENTAL

The cereal products used, with the exception of 2 samples of hard, red spring wheat,[1] were purchased locally in the open market. Their protein contents are given in table 1.

TABLE 1

Protein contents of the cereal products.
The percentages are calculated on an air-dry basis.[1]

MATERIAL	PROTEIN	MATERIAL	PROTEIN
	%		%
Barley, pearled	9.15	Rice, polished	5.24
Corn, whole yellow	9.19	Rice, brown	9.04
Oats, rolled	14.87	Wheat, hard, spring	14.24
Rye, whole	10.96	Wheat, soft, winter	11.02

[1] The following conversion factors were used in calculating the amounts of protein in the materials from their nitrogen content: Wheat, rye, barley and oats, 5.83; corn, 6.25; rice, 5.95 (Jones, '31).

The growth-promoting values of the proteins were determined by feeding experiments with young albino rats. The materials, ground to a fine meal, served as the sole source of protein in diets supplied with the essential non-protein dietary factors. As far as their N contents permitted, the test substances were incorporated in the diets in quantities to supply protein at 4.5, 7.5, 9.5 and 12% levels. Because of their

[1] One of the hard, red spring wheat samples was obtained from the Agricultural Station, Dickenson, North Dakota. The other sample was a Montana wheat supplied through the kind offices of Dr. F. L. Dunlap, of the Wallace Tiernan Company, Chicago, Illinois.

low N contents, not all of the materials could be used at the higher levels without displacing some of the essential accessories. Polished rice, for instance, could be introduced into the diet at only the 4.5% protein level. Only hard wheat and rolled oats permitted comparison at the 12% protein level. The average net gain in weight of each group of animals over a period of 6 weeks and the average gain in weight per gram of protein consumed were used as standards of comparison for evaluating the proteins of the materials studied.

All diets contained 2% cod liver oil and 2% salt mixture (Hubbell, Mendel and Wakeman, '37). The remainder of each of the diets consisted of one of the protein sources listed in table 1, corn oil 8%, and sufficient dextrinized cornstarch to adjust the protein content to the desired levels. A vitamin mixture was incorporated in the dextrinized starch which provided the following constituents in milligrams per 100 gm of diet: thiamine hydrochloride, 0.2; pyridoxine hydrochloride, 0.2; riboflavin, 0.3; calcium pantothenate, 0.3; niacin, 1; and choline hydrochloride, 10. These accessories, in aqueous-alcoholic solution, were added to the dextrinized starch and the mixture was dried at 50°C. to the original weight of the starch.

The data presented represent closely-agreeing results obtained in 2 sets of experiments conducted 1 year apart in which were employed 21 lots of albino rats comparable in all respects. Most of the lots consisted of 20 animals each, equally divided with respect to sex and having initial weights of 45 to 60 gm. They were housed in individual cages having wide mesh screen bottoms and kept in an air-conditioned room maintained at 76° ($\pm$ 2°)F. The animals were weighed and fed ad libitum twice weekly. Fresh diets were prepared in kilogram quantities each week.

RESULTS AND DISCUSSION

The average weight gains of the animals are given in table 2. The nutritive values of the proteins of the grains, compared both with respect to body weight gains and gains

per gram of protein consumed, were found in general to rank in the same order when determined by both procedures.

The order of values of some of the grains as compared with each other varied somewhat with the different protein levels at which they were incorporated in the diets. Oats, rice and rye, however, at each of the protein levels at which they were used, proved superior to all of the other grains.

TABLE 2

Comparative growth-promoting value of the proteins of several cereal grains fed as the sole source of protein in the diet. (Tests conducted by the ad libitum method of feeding over a period of 6 weeks.)

MATERIAL	AVERAGE [1] WEIGHT GAINS	AVERAGE GAIN/GM PROTEIN CONSUMED	AVERAGE FOOD CONSUMED	AVERAGE WEIGHT GAINS	AVERAGE GAIN/GM PROTEIN CONSUMED	AVERAGE FOOD CONSUMED
	gm	*gm*	*gm*	*gm*	*gm*	*gm*
	4.5% protein in diet			7.5% protein in diet		
Corn, yellow	18 ± 2.49	1.42	279	40 ± 2.49	1.56	367
Wheat, hard	18 ± 0.94	1.72	234	43 ± 2.11	1.55	362
Wheat, soft	20 ± 1.32	1.74	216	28 ± 1.68	1.21	326
Barley, pearled	19 ± 1.89	1.55	266	39 ± 2.22	1.67	324
Rice, brown	24 ± 1.50	1.92	288	74 ± 2.30	2.31	418
Rice, polished	33 ± 2.25	2.22	339	..	..	..
Rye	35 ± 2.40	2.26	337	60 ± 2.29	2.16	381
Oats, rolled	38 ± 2.10	2.23	369	59 ± 2.47	2.12	358
	9.5% protein in diet			12% protein in diet		
Wheat, hard	68 ± 2.65	1.60	444	76 ± 2.70	1.47	369
Wheat, soft	66 ± 2.35	1.68	414	..	..	..
Rye	74 ± 2.44	1.83	434	..	..	..
Oats, rolled	99 ± 2.76	2.48	414	118 ± 5.64	2.25	428

[1] Including the probable error of the mean result calculated according to the formula $\sqrt{\Sigma d^2/n - 1/\sqrt{n}} \times 0.6745$, where "d" is the deviation from the mean and "n" is the number of observations (Sherman, '37).

No significant difference was observed between hard and soft wheat, except at the 7.5% protein level. At this level the hard wheat diet supported an average weight gain of 43 gm as compared with 28 gm for the soft wheat. Statistical analysis of the data shows that this difference is significant. The ratio of the mean difference in growth to the probable error of

the difference is 5.5. This implies that the chances are more than 1,300 to 1 that the difference was not fortuitous.

At the 4.5% protein level, corn, hard wheat, soft wheat, barley and brown rice showed essentially the same values. Statistically significant greater gains, however, were made on the oats, rye and white rice diets. At this level white rice showed a significantly higher value than brown rice, and about equaled rye. At the 7.5% level brown rice surpassed rye and oats by odds of 142 to 1. Oats and rye showed values significantly higher than those for hard wheat, barley and corn.

Although at the 9.5% protein level the animals fed rye showed somewhat greater gains than did those that received hard and soft wheat, the differences are not statistically significant.

The superiority of oats, compared with hard and soft wheat at the 9.5% protein level, proved to be highly significant. The oat diet at this level supported an average weight gain of 99 gm as compared with 74 gm for rye, the difference representing odds greater than 100,000 to 1 in favor of oats.

The animals receiving the oat diet at 12% protein level showed an average weight gain of 118 gm as compared with 76 gm gained by those on the hard wheat regime. A statistical analysis of the data shows that the ratio of the mean difference in weight gain to the probable error of the difference is $\pm$ 6.7. With $\pm$ 6E the chances are 19,200 to 1 that another random observation or the mean of any equal random sample will fall within the range 6E.

It is unfortunate that all of the cereals could not have been compared at the higher protein levels. It has been our experience that, in general, optimum growth of young rats fed with plant protein foods is attained at 12 to 15% protein levels in the diet, and that satisfactory comparisons can be made when the material is fed at 10 to 12% levels. Some of the variations in the order of values found when the materials were fed at the low protein levels of 4.5 and 7.5% as compared at the higher levels can well be attributed to borderline

deficiencies in one or more essential amino acids which were supplied in large quantities at the higher protein levels.

A compilation of the amino acid contents of the cereal grains investigated is given in table 3. A strict correlation between amino acid composition and growth-promoting values of the cereals used at the different protein levels cannot be satisfactorily made. Several factors may be involved besides the percentages of amino acid present, such as possible interrelationships between both essential and non-essential amino

TABLE 3[1]

Percentages of amino acids calculated to 16% nitrogen.

	ROLLED OATS	RYE	RICE, WHITE	WHEAT	BARLEY	CORN
Nitrogen	2.73	1.98	1.26	3.07	1.86	2.22
Arginine	7.4	5.4	8.7	4.5	4.5	4.7
Histidine	2.2	2.2	2.3	2.0	1.8	2.2
Lysine	3.0	3.3	2.8	2.5	2.4	2.3
Tryptophane	1.3[2]	1.3[2]	1.3[2]	1.4[2]	1.1[4]	0.5[4]
Leucine	6.5[4]	6.2[2]	7.7[2]	6.8[2]	5.5[4]	15.0[4]
Isoleucine	4.2[4]	4.0[2]	5.1[2]	3.6[2]	3.8[4]	6.4[4]
Valine	5.3	5.0	6.3	4.1	5.1	5.3
Phenylalanine	4.6	3.0	4.6	3.8	5.7	4.8
Threonine	3.6	3.9	3.6	3.0	3.6	3.9
Methionine	1.0	1.1	1.4	1.0	1.0	1.4

[1] With the exceptions indicated, the above values were determined microbiologically in this laboratory by Dr. M. J. Horn and Mr. A. Blum.

[2] Block and Bolling ('45).

[3] Stokes et al. ('45).

[4] Baumgarten et al. ('46).

acids, and the varying threshold limits of amino acids at the different protein levels at which the materials were fed. Mitchell and Block ('46) have pointed out that the amino acid make-up of a protein may not be the only considerable factor limiting its utilization within the animal body. The figures given in the table indicate that oats, rice and rye excel with respect to their contents of lysine and arginine, and that they are surpassed in isoleucine only by corn. Rice, containing the highest percentage of arginine and valine, leads also, with

the exception of corn, in leucine and isoleucine. Corn is characterized by its low tryptophane content and high values for leucine and isoleucine. All of the cereals studied show marked similarity with respect to their histidine and methionine values; and, with the exception of corn, with respect to tryptophane as well.

SUMMARY

The growth-promoting values of the proteins of whole yellow corn, hard and soft wheat, pearled barley, brown and polished rice, rye and rolled oats were compared by means of ad libitum feeding experiments with young albino rats. As far as their N contents permitted, the materials were incorporated at 4.5, 7.5, 9.5 and 12% protein levels in diets approximately isocaloric and nutritionally adequate with respect to dietary factors other than protein.

The results were evaluated on the basis of weight increases and gains per gram of protein consumed over 6-week periods.

At the 4.5% protein level the values ranged in the following descending order: Oats, rye, polished rice, brown rice. Corn, barley, hard and soft wheat showed about the same value, which was lower than that of the other cereals.

Brown rice surpassed all of the others at the 7.5% level. Rolled oats and rye gave much higher values than corn, wheat and barley.

At the 9.5% level oats outranked wheat and rye. Hard and soft wheat gave practically the same values.

A strikingly higher value was obtained for oats than for hard wheat at the 12% protein level — the only two that could be compared at this level.

Oats, rice and rye proved superior to all of the other cereals at the protein levels at which they could be compared.

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THE NUTRITIVE VALUE OF CEREAL PROTEINS IN HUMAN SUBJECTS[1]

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Large quantities of cereals are used directly as human food in this country. For example, in 1944, 46 million bushels of oats and 12 million bushels of corn were consumed as breakfast cereals (U. S. D. A., '46, pp. 46, 56). In the 5-year period 1936–1941 the average annual per capita consumption of breakfast cereals was almost 10 pounds (9.6 lbs.) (U. S. D. A., '42, p. 657).

Several studies of the nutritive value of cereal proteins have been made with human subjects (Mendel and Fine, '11a, b, c; Murlin and Mattill, '38; Murlin, Nasset and Marsh, '38). The latter authors concluded that certain types of heat treatment, particularly the explosion or puffing process, decrease the nutritive value of cereal proteins, as measured by their ability to maintain nitrogen equilibrium. In these studies the cereals were compared with egg protein as the standard protein. In the present investigation certain oat, corn and wheat cereals were compared with the unprocessed cereal with respect to their ability to maintain nitrogen equilibrium in the adult human.

[1] These studies were aided by a grant from the Nutrition Department of General Mills, Inc.

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EXPERIMENTAL

Nitrogen balances were measured on adult human subjects consuming diets the protein of which was furnished primarily by cereals. From the change in the balances, replacement values were calculated by the method of Murlin and Mattill ('38).

The materials used for this study were:

1. Rolled oats,[3] cooked as directed on the package and eaten immediately.

2. An exploded, ready-to-eat cereal,[4] composed of 70% oat flour and 20% corn and rye flours, eaten as it comes from the package. This product will hereafter be referred to as exploded oat cereal.

3. Unprocessed whole wheat. The wheat used was hard red spring wheat which was cracked at the mill and then ground at the laboratory. It was cooked with water and salt in the autoclave for 1 hour at 15 lb. steam pressure.

4. Wheat flakes,[5] eaten as they come from the package.

5. Exploded wheat.[6] Some difficulty was encountered in consuming the large volume of this product until the subjects found it possible to "de-explode" the cereal with water and slight pressure.

6. Yellow hybrid field corn. The entire lot of corn was washed, dried for several days in a stream of warm air and thoroughly mixed. It was stored in air-tight containers in the refrigerator until ready for use. Before eating, the corn was either ground and cooked with water and salt, or cooked with water and salt without grinding. The cooking was done in the autoclave for 1 hour at 15 lb. steam pressure.

7. An oven-expanded, ready-to-eat corn cereal.[7] This product was eaten as it came from the package.

[3] Quaker Oats, manufactured by the Quaker Oats Company.

[4] Cheerioats, produced by General Mills, Inc. This product is now known as Cheerios.

[5] Wheaties, made by General Mills, Inc.

[6] Quaker Puffed Wheat Sparkies, made by Quaker Oats Company.

[7] Kix, made by General Mills, Inc.

8. Corn flakes,[8] eaten as it came from the package.

All of these products, with the exception of the whole wheat and the corn, were purchased from wholesale grocers in Cleveland. The wheat was purchased from the Montana Flour Mills in Cleveland, and the corn from Kohl and Sons Feed Store, Parma Heights, Ohio. Analyses of the cereals used are shown in table 1.

Vitamins were supplied by multiple vitamin capsules,[9] 1 capsule daily furnishing: vitamin A 5000 U.S.P. units, vitamin

TABLE 1

Analyses of products used.

PRODUCT	MOISTURE	NITROGEN
	%	%
Milk-cream mixture	....	0.47
Rolled oats	7.90	2.59
Exploded oat cereal	5.62	2.17
Whole wheat	13.38	2.42
Wheat flakes		
Lot 1915	4.58	1.65
Lot 2185	5.83	1.60
Exploded wheat	5.26	2.24
Field corn	12.73	1.36
Expanded corn cereal		
Lot 2845	5.04	1.25
Lot 2955	4.97	1.25
Lot 2965	5.12	1.26
Corn flakes	2.89	1.22

D 500 U.S.P. units, thiamine hydrochloride 1.5 mg, riboflavin 2 mg, nicotinamide 20 mg, pyridoxine hydrochloride 0.5 mg, calcium pantothenate 5 mg, and ascorbic acid 37.5 mg. On the corn diets, the vitamin capsules [10] supplied daily: vitamin A 10,000 U.S.P. units, vitamin D 1000 U.S.P. units, thiamine hydrochloride 5 mg, riboflavin 5 mg, nicotinamide 25 mg, pyridoxine hydrochloride 1.5 mg, calcium pantothenate 5 mg,

[8] Kellogg Corn Flakes, made by the Kellogg Company.

[9] Abbott's Vitakaps Improved, made by Abbott Laboratories.

[10] Abbott's Dayamins, made by Abbott Laboratories.

and ascorbic acid 100 mg. Salt and water were allowed ad libitum.

On the first 2 experiments with rolled oats and exploded oat cereal, additional protein was supplied as a 1:1 mixture of milk and cream (20%). Calories needed in addition to the cereal and milk were supplied by butter (or oleomargarine) or sucrose or both.

The diets were designed to provide 0.70 gm of protein ($N \times 6.25$) and approximately 37.5 Cal. per kg of body weight per day. In the first experiment on rolled oats and exploded oat cereal, the cereal supplied approximately 55% of the total nitrogen intake. In the second experiment on the same cereals, the cereal nitrogen supplied 80% of the total dietary nitrogen. In the remainder of the experiments, the cereal supplied, as nearly as possible, all of the dietary nitrogen. The diets are summarized in table 2.

A total of 8 subjects (3 women, 5 men) were used in these studies. They were all white adults, all except one having had training in biochemistry, and all aware of the importance of accurate and complete urine and stool collections and careful weighing of the daily diets. They cooperated well in consuming the prescribed diets, in abstaining completely from food and drinks not prescribed, and in reporting the almost inevitable occasional loss of part of a urine specimen. Throughout the experiments the subjects continued in their normal activities.

The diets were eaten for a period of 2 days before collections were begun. On the morning that collections were started, a stool marker (charcoal or barium sulfate) was ingested before breakfast, and urine collections begun. Stool collections were started when the marker appeared, including the marked stool with the collection. At the end of the experimental period, another marker was ingested on the morning that the last urine sample was completed and stool collections continued until the marker appeared, not including the marked stool with the collections. The subjects were

TABLE 2

Summary of daily diets.

SUBJECT	CEREAL	MILK-CREAM MIXTURE	SUCROSE	BUTTER	NITROGEN
	gm	*ml*	*gm*	*gm*	*gm*
Exp. 1	Exploded oat cereal				
G.G.	153	628	206	0	6.26
L.G.	136	554	183	0	5.55
R.L.	160	646	215	0	6.51
C.K.	207	834	280	0	8.41
W.P.	157	634	212	0	6.38
M.P.	189	768	253	0	7.71
	Rolled oats				
G.G.	152	628	204	0	6.88
L.G.	135	554	181	0	6.10
R.L.	159	646	213	0	7.15
C.K.	206	834	276	0	9.25
W.P.	156	634	210	0	7.02
M.P.	187	768	251	0	8.45
Exp. 2	Exploded oat cereal				
G.G.	237	272	223	0	6.42
L.G.	212	246	200	0	5.75
R.L.	250	292	234	0	6.80
C.K.	317	366	295	0	8.59
W.P.	240	276	227	0	6.49
G.S.	291	336	274	0	7.89
	Rolled oats				
G.G.	199	272	250	0	6.42
L.G.	178	246	224	0	5.75
R.L.	210	292	262	0	6.80
C.K.	266	366	332	0	8.59
W.P.	201	276	254	0	6.49
G.S.	244	336	306	0	7.89
Exp. 3	Exploded oat cereal				
A.F.	436	0	327	0	9.46
C.K.	388	0	177	80	8.42
G.S.	360	0	38	142	7.81
	Rolled oats				
A.F.	366	0	375	0	9.46
C.K.	325	0	222	80	8.42
G.S.	302	0	76	142	7.81
Exp. 4	Whole wheat				
A.F.	404	0	390	0	9.80
C.K.	356	0	194	113	8.63
G.S.	361	0	184	120	8.75
	Wheat flakes 1915				
A.F.	594	0	197	0	9.80
C.K.	523	0	0	125	8.63
G.S.	530	0	0	125	8.75
	Wheat flakes 2185				
A.F.	612	0	180	0	9.80
C.K.	540	0	0	117	8.63
G.S.	547	0	0	117	8.75
	Exploded wheat				
A.F.	437	0	345	0	9.80
C.K.	385	0	151	114	8.63
G.S.	391	0	96	150	8.75
Exp. 5	Field corn				
A.F.	721	0	93	0	9.80
C.K.	635	0	0	76	8.63
G.S.	644	0	142	0	8.75
	Expanded corn cereal				
A.F.	784	0	45	0	9.80
C.K.	690	0	0	55	8.63
G.S.	700	0	0	53	8.75
	Corn flakes				
A.F.	804	0	27	0	9.80
C.K.	707	0	0	46	8.63
G.S.	717	0	84	0	8.75

weighed daily after completing a 24-hour urine sample and before breakfast.

The urines were collected directly into bottles containing toluene as a preservative. The 24-hour samples were mixed, volume and specific gravity measured, and an aliquot removed for analysis. The stool collections were made directly into wide mouth mason jars having lids sealed with rubber rings. All samples were kept under refrigeration until analyzed.

The urine samples were analyzed for total nitrogen and creatinine. Total nitrogen determinations were run by a semi-micro Kjeldahl distillation-titration procedure, using copper sulfate or selenium, or both, as catalyst. Urine creatinine was measured by an adaptation of the Folin ('14) colorimetric procedure to the Evelyn photoelectric colorimeter. In those urine samples, reported as being incomplete, in which the creatinine content showed a marked deviation from the mean daily creatinine excretion of that subject, a correction was applied. The actual nitrogen figure obtained by analysis was multiplied by the ratio mean daily creatinine/creatinine in sample to estimate the total nitrogen in the complete sample. In a total of 462 daily urine collections, there were 4 incomplete samples, of which 3 occurred in the first experiment.

The stool specimens for each subject were pooled, partially digested with sulfuric acid on the steam bath, cooled, made up to volume and sampled with mixing (Peters and Van Slyke, '32). Total nitrogen was estimated as with the urine samples.

RESULTS AND DISCUSSION

In the first experiment 3 subjects (L.G., M.P., W.P.) started on the exploded oat cereal diet (table 2) and 3 subjects (G.G., R.L., C.K.) started on the rolled oats diet (table 2). These diets were continued until collections had been made for 9 days, at which time a stool marker was ingested and the 2 groups changed diets, those who had been eating the exploded oat cereal substituting rolled oats and *vice versa*. Urine and stool collections were continued for 9 more days. The mean daily nitrogen balances observed in these subjects are pre-

sented in the upper part of table 3. It will be seen that in every case the nitrogen balance is more favorable on the rolled oats diet than it is on the exploded oat cereal diet. From this, it might be inferred that the availability of the nitrogen of the exploded oat cereal was less than that of rolled oats. However,

TABLE 3

Mean daily nitrogen balances (all figures are grams of nitrogen per day).

SUBJECT [1]	EXPLODED OAT CEREAL				ROLLED OATS			
	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance
	A. Experiment 1. Cereal furnished 55% of dietary nitrogen.							
G.G.	6.26	5.15	1.49	−0.38 ± .48 [2]	6.88	5.87	1.32	−0.31 ± .33 [2]
R.L.	6.51	5.50	0.87	+0.14 ± .54	7.15	5.23	0.82	+1.10 ± .69
C.K.	8.41	8.52	1.31	−1.42 ± .85	9.25	9.13	1.24	−1.12 ± .42
L.G.	5.55	6.36	0.84	−1.64 ± .29	6.10	5.66	1.06	−0.62 ± .58
M.P.	7.71	7.24	1.47	−1.00 ± .83	8.45	6.92	1.52	+0.01 ± .58
W.P.	6.38	7.25	1.42	−2.29 ± .69	7.02	6.55	1.59	−1.12 ± .38
Mean	6.80			−1.10	7.47			−0.34
	B. Experiment 2. Cereal furnished 80% of dietary nitrogen.							
G.G.	6.42	5.12	1.51	−0.21 ± .29	6.42	5.97	1.40	−0.95 ± .16
R.L.	6.80	5.03	1.87	−0.10 ± .31	6.80	5.21	0.73	+0.86 ± .31
C.K.	8.59	7.05	1.93	−0.39 ± .27	8.59	7.36	1.47	−0.24 ± .44
L.G.	5.75	5.65	0.68	−0.58 ± .27	5.75	6.53	1.45	−2.23 ± .31
G.S.	7.89	8.23	1.81 [3]	−2.15 ± .57	7.89	6.40	1.89	−0.40 ± .29
W.P.	6.49	7.42	1.50 [3]	−2.43 ± .55	6.49	6.61	1.50	−1.62 ± .37
Mean	6.99			−0.98	6.99			−0.76

[1] The sex, age, height (cm), weight (kg), respectively, of the subjects are as follows: G.G., F, 29, 161, 57.2; L.G., F, 29, 167, 51.5; R.L., F, 21, 163, 60.7; C.K., M, 29, 181, 77.5; W.P., M, 37, 173, 58.2; M.P., M, 29, 170, 72.9; G.S., M, 26, 186, 74.8; A.F., M, 31, 175, 85.6.

[2] Standard deviation of mean.

[3] Estimated from digestibility as shown in table 4.

in every case the nitrogen intake was also higher on the rolled oats diet than it was on the exploded oat cereal diet, and all of the increased intake represented rolled oats nitrogen. The more favorable balance (mean 0.76 gm nitrogen per day) on the rolled oats diet was undoubtedly due, at least in part, to the increased nitrogen intake (mean 0.67 gm nitrogen per

day) on the rolled oats diet. The data thus obtained do not permit any definite conclusion with regard to the relative utilization of the nitrogen of these 2 cereals.

On the basis of the data reported by Stewart et al. ('43) on growing rats, it was expected that a large difference in the utilization of the nitrogen of these 2 products would be apparent. Since inconclusive results were obtained on the first experiment, it was decided to repeat the experiment with more

TABLE 4

Experiment 2.

Estimation of fecal nitrogen for G.S. and W.P. on exploded oat cereal diet.

SUBJECT	DRY MATTER INTAKE [1]	METABOLIC NITROGEN [2]	FECAL NITROGEN	DIGESTIBILITY [3]	ESTIMATED FECAL NITROGEN
	gm	*gm*	*gm*	*%*	*gm*
G.G.	504	1.16	1.51	94.5	...
R.L.	531	1.22	1.87	90.5	...
C.K.	671	1.54	1.93	95.5	...
L.G.	452	1.04	0.68	100	...
G.S.	619	1.42	...	...	1.81
W.P.	511	1.18	...	...	1.50
				Mean 95.1	

[1] Dry matter calculated using the data of tables 1 and 2 and assuming 21% solids in the milk-cream mixture.

[2] Fecal metabolic nitrogen calculated assuming 0.23 gm per 100 gm dry matter consumed (Mitchell and Hamilton, '29, p. 234).

[3] Per cent digestibility $= \frac{[\text{Food N} - (\text{Fecal N} - \text{Metabolic N})]}{\text{Food N}} \times 100$. (Hawk et al., '47, p. 961.)

of the protein of the diet being supplied by the cereal and less from the milk-cream mixture.

The plan of the second experiment was the same as that of the first, except that the cereal supplied 80% of the dietary nitrogen and the experimental period was shortened to 4 days' collection on each diet. Three of the subjects (L.G., W.P., G.S.) started on the exploded oat cereal diet, and 3 (G.G., R.L., C.K.) started on the rolled oats diet.

The mean daily nitrogen balances observed are presented in the lower part of table 3. In the case of subjects G.S. and

W.P., the stool specimens on the exploded oat cereal diet were lost before they had been analyzed, and consequently the fecal nitrogen for these 2 subjects had to be estimated indirectly. This was done by making use of the digestibility of the exploded oat cereal-milk protein mixture calculated from the intakes of the other 4 subjects as shown in table 4. It will be seen that in 4 of the 6 subjects, the nitrogen balance on rolled oats and milk was more favorable than the nitrogen balance on exploded oat cereal plus milk, but the mean replacement value (table 5) shows a very slight difference between the 2

TABLE 5

Experiment 2.

Calculation of replacement value.

SUBJECT	NITROGEN BALANCE ON		DIFFERENCE IN BALANCE	NITROGEN INTAKE	REPLACEMENT VALUE
	Rolled oats + milk	Exploded oats + milk			
	(1)	(2)	(3)[1]	(4)	(5)[2]
G.G.	— 0.95	— 0.21	+ 0.74	6.42	112
R.L.	+ 0.86	— 0.10	— 0.96	6.80	86
C.K.	— 0.24	— 0.39	— 0.15	8.59	98
L.G.	— 2.23	— 0.58	+ 1.65	5.75	129
G.S.	— 0.40	— 2.15	— 1.75	7.89	78
W.P.	— 1.62	— 2.43	— 0.81	6.49	88
				Mean	98.5

[1] Column (2) minus column (1).

[2] Column (3) expressed as a per cent of column (4). If column (3) is positive, the per cent is added to 100; if negative, subtracted from 100.

protein mixtures. It is thus doubtful if there is any real difference in availability of the protein of exploded oat cereal plus milk as compared with that of rolled oats plus milk.

There still being some doubt in our minds with regard to the significance of the results, it was decided to try another experiment in which the supplementing effect, if any, of the milk protein was absent. Three white adult male subjects (A.F., C.K., G.S.) were put on diets (table 2) in which all of the protein was furnished by the cereal. The exploded oat cereal diets were eaten for a total of 12 days, on the last 10 of

which urine and stool collections were made.[11] At the end of the 12-day period, a stool marker was ingested and a daily supplement of 5 gm DL-lysine monohydrochloride included in the diet for an additional 5-day period. The lysine was mixed with the food after cooking so that the supplement would be taken at the same time as the food and not in a single dose. Berg and Rose ('29) have observed that amino acid supplements are more effective if taken in divided doses, rather than in a single dose. After a rest period of 11 days, exactly the same procedure was applied to rolled oats. Lysine was chosen as the amino acid supplement because it is the first limiting essential amino acid in these cereals.[12]

The results are summarized in the first part of table 6. In every case, the addition of lysine to the exploded oat cereal diet improved the nitrogen balance, the mean increase being 1 gm per day. This would appear to indicate that the amount of the exploded oat cereal consumed furnished insufficient lysine for the maintenance of nitrogen equilibrium in adult human subjects. In the same way, addition of lysine to the rolled oats diet increased the nitrogen balance an average of 0.96 gm per day. The rolled oats, in the quantities consumed, would therefore also appear to furnish insufficient lysine for nitrogen equilibrium in adult humans.

The replacement values are calculated as shown in table 7. It would seem that the replacement value of exploded oat cereal nitrogen, in terms of rolled oats nitrogen (101%), indicates no significant difference in the utilization of the nitrogen of these 2 cereals by the adult human when they are consumed in quantities to furnish 0.112 gm nitrogen per kg of body weight per day. Similarly, the replacement value of exploded oat cereal plus lysine, in terms of rolled oats plus lysine (101%), when ingested in these quantities, would ap-

[11] In the case of subject G.S., the figures used represent only the last 7 days of collection, since on the third day of collection he had a gastrointestinal upset and was unable to eat his full quota of food. On the morning of the fourth day, he ingested another marker to separate the stool of the first 3 days from that of the remainder of the experiment.

[12] Personal communication from Dr. Lela E. Booher, General Mills, Inc.

TABLE 6

Summary of mean daily nitrogen balances (all figures are grams of nitrogen per day).

DIET	A.F.			C.K.			G.S.			MEAN BALANCE
	Urine	Stool	Balance	Urine	Stool	Balance	Urine	Stool	Balance	
Exploded oat cereal	9.24	2.12	−1.90 ± .92[1]	7.13	1.49	−0.20 ± .35[1]	7.33	1.88	−1.40 ± .99[1]	−1.17
Exploded cereal + lysine	8.40	2.32	−0.50 ± .87	7.06	1.71	+0.41 ± .23	6.96	2.03	−0.42 ± .33	−0.17
Rolled oats	8.93	2.59	−2.06 ± 1.06	7.13	1.67	−0.38 ± .52	6.69	2.33	−1.21 ± .32	−1.22
Rolled oats + lysine	8.43	2.46	−0.67 ± .80	7.45	1.65	+0.08 ± .43	6.78	1.97	−0.18 ± .91	−0.26
Whole wheat	8.63	2.67	−1.50 ± .71	7.02	1.95	−0.34 ± .35	7.03	2.02	−0.30 ± .50	−0.71
Wheat flakes	9.15	2.51	−1.74 ± .58	7.32	2.25	−0.94 ± .45	7.36	2.29	−0.90 ± .32	−1.19
Exploded wheat	11.24	2.49	−3.92 ± .73	7.52	2.28	−1.17 ± .55	7.75	2.56	−1.56 ± .50	−2.22
Field corn	7.87	3.64	−1.71 ± .69	6.64	3.59	−1.60 ± .57	7.71	2.52	−1.48 ± .65	−1.60
Expanded corn cereal	8.99	3.31	−2.50 ± .65	6.99	2.57	−0.93 ± .33	7.05	2.93	−1.22 ± .55	−1.55
Corn flakes	8.78	3.39	−1.74 ± .74	7.15	2.77	−1.28 ± .48	7.24	2.89	−1.38 ± .53	−1.67

[1] Standard deviation of mean.

TABLE 7

Calculation of replacement values.

SUBJECT	BALANCE ON		DIFFERENCE [1]	NITROGEN INTAKE	REPLACEMENT VALUE [2]
A Oat cereals					
	Rolled oats	Exploded oats			
A.F.	— 2.06	— 1.90	+ 0.16	9.46	102
C.K.	— 0.38	— 0.20	+ 0.18	8.42	102
G.S.	— 1.21	— 1.40	— 0.19	7.81	98
				Mean	101
	Rolled oats + lysine	Exploded oats + lysine			
A.F.	— 0.67	— 0.50	+ 0.17	10.22	102
C.K.	+ 0.08	+ 0.41	+ 0.33	9.18	104
G.S.	— 0.18	— 0.42	— 0.24	8.57	97
				Mean	101
B Wheat cereals					
	Whole wheat	Wheat flakes			
A.F.	— 1.50	— 1.74	— 0.24	9.80	98
C.K.	— 0.34	— 0.94	— 0.60	8.63	93
G.S.	— 0.30	— 0.90	— 0.60	8.75	93
				Mean	95
	Whole wheat	Exploded wheat			
A.F.	— 1.50	— 3.92	— 2.42	9.80	75
C.K.	— 0.34	— 1.17	— 0.83	8.63	90
G.S.	— 0.30	— 1.56	— 1.26	8.75	86
				Mean	84
C Corn cereals					
	Field corn	Expanded corn			
A.F.	— 1.71	— 2.50	— 0.79	9.80	92
C.K.	— 1.60	— 0.93	+ 0.67	8.63	108
G.S.	— 1.48	— 1.23	+ 0.25	8.75	103
				Mean	101
	Field corn	Corn flakes			
A.F.	— 1.71	— 2.36	— 0.65	9.80	93
C.K.	— 1.60	— 1.28	+ 0.32	8.63	104
G.S.	— 1.48	— 1.38	+ 0.10	8.75	101
				Mean	99

[1] [2] See footnotes to table 5.

pear to indicate no significant difference in the utilization of these nitrogen sources by adult human subjects.

Supplementation of the 2 oat cereal diets with lysine brings about an improvement in the nitrogen balance in each case, amounting to from 0.46 to 1.40 gm of nitrogen per day. Five grams of DL-lysine monohydrochloride contains 0.76 gm of nitrogen. If it is assumed that in the human, as in the rat, only the L-isomer is physiologically available (Berg, '36), then the addition of 0.38 gm of nitrogen as L-lysine brought about the retention of from 0.08 to 1.02 gm of "extra" nitrogen (or from 21 to 268%) in addition to that of the L-lysine.

The mean improvement in the nitrogen balance on the addition of lysine was very nearly the same whether the lysine was added to the exploded oat cereal diet or the rolled oats diet. This would seem to indicate that the degree of lysine deficiency was approximately the same on the exploded oat cereal diet as on the rolled oats diet. Amino acid analyses (Mitchell and Block, '46, table 9) show the 2 cereals to have about the same lysine content.

It would thus appear that the processing of the exploded oat cereal had no significant effect on the nutritive value of the nitrogen of this cereal so far as adult human subjects are concerned.

The similarity in nutritive value of the proteins of these 2 oat cereals appears to be in disagreement with the findings reported by Stewart et al. ('43). These workers observed, in growing white rats, only 32 to 39% as good gain in weight per gram of protein eaten with the exploded oat cereal as with rolled oats. There are 2 important differences between the present study and that of Stewart et al. In the present study *adult human* subjects were used, whereas Stewart et al. used *growing* albino *rats*.

It is quite generally conceded that the nutritive value of a protein or mixture of proteins depends upon the mixture of amino acids made available to the organism as a result of the ingestion of that protein (see e.g., Jackson, '45, p. 987). It is conceivable that there is a quantitative difference in the

amino acid requirements for growth and for maintenance (Jackson, '45, p. 991; Neuberger and Webster, '45), and also there may well be a qualitative difference (Almquist, '45, p. 1271). In light of this observation along with a consideration of species differences in amino acid requirements (see e.g., Womack and Kade, '44, pp. 223–229), the results of the 2 studies may not be in radical disagreement. The depressed nutritive value, for the growing rat, of the protein of exploded oat cereal as compared with rolled oats (Stewart et al., '43; Mitchell and Block, '46) apparently is due to some factor other than the amino acid content. Mitchell and Block ('46) suggest several possible explanations. In any case, the ultimate criterion of nutritive value for humans must be the results of studies done on human subjects.

In the fourth experiment the same 3 subjects were used for a comparison of the nitrogen balances on 2 prepared wheat cereals and unprocessed whole wheat. The diets (table 2) were eaten for a total of 14 days each, with complete urine and stool collections for the last 12 days.[13] The exploded wheat diet was eaten first, followed, after a rest period of a week, by the wheat flakes diet. After another rest period of 11 days, the whole wheat diet was eaten.

The observed nitrogen balances are summarized in table 6. It will be seen that, with all of the 3 subjects, the most favorable balance was observed on the whole wheat diet, there was an intermediate value on the wheat flakes diet, and the most unfavorable balance was observed on the exploded wheat diet. The difference between the mean balance on wheat flakes and the mean balance on whole wheat (0.48) was only a third as great as the difference between the mean balance on the exploded wheat and the mean balance on the whole wheat (1.51). The replacement values (table 7, section B) also show this difference between the 2 processed wheat cereals. It would

[13] In the case of subject A.F. on the wheat flakes diet, the figures for only 11 days were used, since on one of the days he had gastrointestinal upset with diarrhea and vomiting, and did not eat his full quota of food. The following morning he felt equal to continuing the experiment and ingested an extra fecal marker to separate the stool for the 2 periods.

appear that the processing brought about a greater decrease in the nutritive value of the nitrogen in the exploded wheat than in the wheat flakes. These findings are in agreement with the results reported by Murlin, Nasset and Marsh ('38) who found the protein of exploded wheat to be definitely inferior to that of wheat flakes.

The final experiment was a comparison of the nitrogen balances of the same 3 subjects on 2 processed corn cereals and unprocessed whole corn. The first diet to be eaten was the expanded corn cereal diet, which was followed by the corn flakes diet after a rest period of about a month. This was

TABLE 8

Summary of nitrogen balances for 3 subjects (all figures are grams of nitrogen per day).

CEREAL	MEAN N INTAKE	MEAN N BALANCE
Whole wheat	9.06	— 0.71
Exploded oat cereal[1]	8.56	— 1.17
Wheat flakes	9.06	— 1.19
Rolled oats[1]	8.56	— 1.22
Expanded corn cereal	9.06	— 1.55
Whole corn	9.06	— 1.60
Corn flakes	9.06	— 1.67
Exploded wheat	9.06	— 2.22

[1] The position of the oat cereals in the table is probably influenced by the nitrogen intake. See text for discussion.

followed, after a rest period of from 23 to 52 days, by the whole corn diet.

The mean daily nitrogen balances observed are shown in table 6. The mean balance for the 3 subjects was best on the expanded corn cereal diet, intermediate on the field corn diet and poorest on the corn flakes diet. The replacement values (table 7, section C) indicate little, if any, significant difference in the nitrogen of these 3 cereals in their ability to maintain nitrogen equilibrium in adult human subjects.

An estimate of the relative value of the proteins of the unprocessed cereals can be obtained since, on the last 3 experi-

ments, the same 3 subjects were used. The mean balances are presented in table 8 in decreasing order. Of the unprocessed cereals, it will be seen that in these experiments the best balance was observed on whole wheat, followed by rolled oats and then corn. It is generally accepted that the protein of oats has a higher nutritive value than that of wheat (see, e.g., Wilder and Keys, '42). The variance of the present results may be due to the lower nitrogen intake on the oat cereals, since the difference in nitrogen intake (0.50 gm per day) was equal to the maximum difference in nitrogen balance (0.51 gm per day).

CONCLUSIONS

The following conclusions appear justified on the basis of the data presented.

1. Rolled oats and exploded oat cereal, as sources of nitrogen, are equally well utilized by adult human subjects when they furnish 55, 80 or 100% of the total dietary nitrogen, the remainder of the nitrogen being furnished by milk, when the total protein ($N \times 6.25$) intake is approximately 0.70 gm per kg body weight per day, and the Caloric intake approximately 37.5 Cal. per kg body weight per day.

2. Both of these cereals, at this level of intake, furnish suboptimal amounts of lysine, and the degree of lysine deficiency of the 2 cereals is the same within the limits of error of the method used.

3. Both wheat flakes and exploded wheat are inferior to unprocessed whole wheat in the maintenance of nitrogen equilibrium. The wheat flakes, however, are definitely better in this respect than the exploded wheat.

4. The nitrogen of the expanded corn cereal and corn flakes is practically identical with that of unprocessed whole corn in its ability to maintain nitrogen equilibrium in the adult human.

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THE ROLE OF THYROID ACTIVITY IN THE PATHOGENESIS OF HEPATIC LESIONS DUE TO CHOLINE AND CYSTINE DEFICIENCY

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TWO FIGURES

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INTRODUCTION

The possibility that increased activity of the thyroid gland might have a deleterious effect on the liver in man was first suggested by Paul in 1865. During the intervening years there have been numerous allusions to a thyroid-liver relationship, the exact nature of which has never been very clearly defined, nor have morphological changes in the liver during life (Piper and Paulsen, '47) or at autopsy (Weller, '33) been at all constant. In experimental animals, the administration of thyroid substance or thyroxine at high dosage levels for a relatively short period has not resulted in liver changes other than those usually seen in starvation; moderate enlargement has been observed in longer experiments where lesser amounts of thyroid were administered (McIver, '42).

Choline deficiency in the rat leads to extreme fatty infiltration of the liver, followed by focal areas of necrosis and fibrosis; on the other hand, cystine deficiency results in acute, massive, hepatic necrosis without fatty infiltration (György, '44; Glynn et al., '45; Handler and Dubin, '46). It seemed of

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interest, therefore, to determine whether the effects of choline or cystine deficiency could be modified by varying the level of thyroid activity. That this may occur has already been indicated by the observations of György and Goldblatt ('45) that thiouracil, which interferes with the formation of the active principle of the thyroid, inhibits the development of cirrhosis in choline-deficient rats.

EXPERIMENTAL

The animals used in these experiments were male rats of the Vanderbilt strain (Wolfe, Bryan and Wright, '38). They were placed on the experimental regimes when they had attained a weight of 50 gm, and housed in individual cages. All experiments were terminated after 120 days unless otherwise stated. At this time the animals were sacrificed by carotid section and livers and thyroids removed and preserved in formalin. Blocks were embedded in paraffin; sections were cut and then stained with hematoxylin and eosin.

In estimating the degree of change, i.e., fatty infiltration, scarring and necrosis, all of the sections have been graded arbitrarily 1 to 4 plus on the basis of the degree of alteration present. The final estimation is a summary of the total number of animals in each group. In view of the rather wide variations in morphological changes from animal to animal, such a mode of expression is certainly not ideal. It would seem, however, the only suitable way of summarizing the data and is similar to the procedure used earlier (Handler and Dubin, '46). It should be pointed out that the variations in the response of the liver to choline-deficient diets are notorious. In our experience, based on studies of various mineral and vitamin deficiencies in rats, choline deficiency is unique in the inconstancy of the final morphological picture.

Thyroid activity and hepatic damage in choline-deficient rats

The basal ration used for this study was patterned after that previously found in this laboratory to be most effective

in producing choline deficiency in rats (Handler and Dubin, '46). It had the following percentage composition: casein 5, starch 60, cotton oil 10, lard 15, cod liver oil 5, cystine 0.5, cholesterol 0.5 and salts (Hubbell, Mendel and Wakeman, '37) 4. In addition, the diet contained in milligrams per kilo, the following vitamins: thiamine 3, riboflavin 5, pyridoxine 3, calcium pantothenate 30, niacin 100, naphthohydroquinone acetate 5, biotin 1, folic acid 2, α-tocopherol 50. The additional supplements to this ration and the results are summarized in table 1.

The history of the animals in group A was similar to that of previous rats on such diets (Handler and Dubin, '46). At autopsy the livers of these animals contained large amounts of fat. In addition, there were areas of varying size where the liver cells had become necrotic and had been replaced by connective tissue. In many of the livers, large areas of scarring were encountered so that the picture was not entirely that of nodular cirrhosis as had been encountered before when this diet was employed. Examination of the thyroid glands of this group revealed hyperplasia in 3 of the animals examined.

Hypothyroidism induced by thyroidectomy and by thiouracil or p-aminobenzoic acid feeding appeared to protect rats against the deleterious effects of choline deficiency. The results obtained with the thyroidectomized rats in group B were not very convincing since almost all the animals died before the termination of the experiment, albeit somewhat later than the control rats of group A. Unfortunately, the livers of these animals were so badly autolyzed that satisfactory histological examination was possible in only 2 of this group. Death may have been entirely unrelated to the choline deficiency since a control, choline-fed group, not shown in the table, also died after about 100 days. It does seem significant that no fat, necrosis, or scarring was observed in the 2 livers of group B rats which were suitable for examination. Of the other 2 agents, thiouracil which produced the more extensive hyperplasia of the thyroid was also most effective in protecting

TABLE 1

Thyroid activity and hepatic damage in choline-deficient rats.

GROUP	DIETARY SUPPLEMENTS	NO. RATS	RATS DIED	SURVIVAL TIME [1]	WEIGHT CHANGE	LIVER				THYROID
						Number examined	Fat	Necrosis	Fibrosis	
				days	*gm*					
A	Basal 1	14	12	77	+ 18	8	3 +	3 +	2 +	Hyperplasia ±
B	Thyroidectomized	8	6	93	15	2	+	0	0	..
C	Thyroid 0.3%	8	8	45	3	5	+	±	0	Excess colloid
D	Thiouracil 0.15%	8	2	94	8	8	2 +	+	±	Hyperplasia 3 +
E	p-Aminobenzoic acid 2%	8	0	..	26	8	3 +	2 +	+	Hyperplasia 2 +
F	Taurine 1%	8	1	87	7	8	3 +	2 +	+	Normal
H	Sulfasuxidine 1%	16	0	..	26	8	4 +	2 +	+	Normal
J	Inositol 0.5% Tocopherol 10 mg/day	8	1	81	24	7	3 +	2 +	+	Normal
AA	Basal 2	14	7	63	28	10	3 +	+	0	Normal
CC	Thyroid 0.1%	11	2	85	14	11	2 +	0	0	Excess colloid
CCC	Thyroid 0.3%	7	5	33	7	7	+	0	0	Excess colloid
EE	p-Aminobenzoic acid 2%	9	1	106	41	9	3 +	+	0	Hyperplasia 2 +

[1] Dead rats only.

against choline deficiency. While the livers of these 2 groups were not completely free of necrosis or connective tissue, it is probably most significant that so few animals died during the experimental period. On the other hand, it also remains possible that the hepatic damage would have increased in severity if the experiment had been prolonged further.

In earlier, unpublished studies of the factors involved in ceroid deposition, it was observed that sulfasuxidine also protected against the hepatic damage of choline deficiency. This was repeated in the present series and it was again observed that the incorporation of sulfasuxidine into the basal diet markedly lessened the tendency of choline-deficient livers to become necrotic and scarred. This seems even more impressive when it is noted that these were also the most heavily fatty livers of the entire series, perhaps because of an induced inositol deficiency (Handler, '46a). In conformity with the findings of Astwood et al. ('43), the thyroid glands of this group were essentially normal and it seems unlikely that the mechanism by which this compound protected against hepatic damage is similar to that of thiouracil or p-aminobenzoic acid.

Since the mode of action of sulfasuxidine was not clear, it was thought of interest to include a group of animals offered taurine in a choline-deficient diet, as this substance is one of the few normal metabolites containing sulfur in a comparable oxidized state. As shown in the table, this substance had little effect on liver fat. Nevertheless, it did offer considerable protection against the deleterious effects of the basal diet since only 1 animal died during the experimental period, and all the livers showed less necrosis and fibrosis than did those of group A.

Because of the well-established lipotropic action of inositol and the protection afforded rats against the renal damage of choline deficiency by a combination of inositol and tocopherol (Handler, '46b), it was thought of interest at this time to test the effect of this combination as an addition to the basal ration. As judged histologically, this did not result in any effective diminution in liver fat, but did reduce the severity of the

hepatic necrosis and fibrosis and, perhaps most significant, only 1 of 8 rats died during the experimental period.

The results of thyroid feeding in this, as in the subsequent experiments, were rather inconclusive. All of the animals died, and much sooner than did those on the control diet. However, there was great variation in the amount of fat and necrosis observed in their livers. The rats that died soonest showed very little fat or necrosis, while the livers of the animals living longest contained more fat and were somewhat more necrotic; however, none approached the lipoid content of the controls. Only 1 liver in this series was at all scarred, perhaps because the duration of life on this regime was too brief to permit any appreciable proliferation of fibrous tissue. The thyroid glands in this group, as was to be expected, were large and of the colloid type. Not included in table 1 were several groups of rats fed the basal ration to which considerably more thyroid powder had been added, in amounts up to 0.8% of the diet. These animals all died much more rapidly (7 to 20 days) and their livers showed no abnormalities.

Included also in table 1 are the results obtained when the fat content of the basal diet was lowered. Basal diet 2 contained 5% each of the cotton oil, lard and cod liver oil, a total of 15%, in contrast with basal diet 1 which contained 30% lipids. The relatively large amount of cod liver oil in these diets was deliberately included to enhance the deposition of ceroid (Wachstein, '45). Under these conditions, animals on the unsupplemented basal diet, group AA, were not as severely affected as those on the higher fat intake. Only one-half of the animals died and, while all animals showed very fatty livers, only moderate necrosis resulted and fibrosis was seen in only 1 of the 10 livers examined. Thyroid feeding at 0.1% of the diet did not hasten the demise of the animals, nor was there a differentiation between the livers of these rats and those of the unsupplemented controls. Increasing the thyroid dosage to 0.3% again resulted in early death of the animals but these showed variable amounts of liver fat and virtually no fibrosis or necrosis. And again, p-aminobenzoic acid, in large

amounts, produced morphological thyroid hyperplasia with complete protection against the necrotic and fibrotic processes although the livers were extremely fatty.

Thyroid activity and hepatic damage in cystine-deficient rats

In table 2 are presented the effects of varying levels of thyroid activity on the picture of acute cystine deficiency. Group A received basal ration 2 (low fat) while group B received basal ration 1 (high fat). No cystine was added to either ration. Thus, these 2 groups were both choline- and cystine-deficient. While the animals of group A (with 2 exceptions) died about 2 weeks before those of group B, their livers contained considerably less fat and were only patchily necrotic. In contrast, the necrosis in group B was not focal or central but involved great masses of liver. All further studies were made with the basal ration 1, without cystine, but with the supplements indicated in the table.

The addition of choline did not significantly delay death due to cystine deficiency but the livers of this group (E) did not seem quite as severely affected as those of group B. Thus, when groups A and E are compared with group B, it appears that the hepatic necrosis of cystine deficiency is more pronounced when conditions are such that the liver is concomitantly fatty, all other factors being held as constant as possible. Thyroid feeding accelerated the death of cystine-deficient rats (F) and also that of animals deficient in both cystine and choline (C). Both of these groups presented the dramatic picture of acute massive necrosis found in control group B even though they died several weeks earlier. This is in contrast to the behavior of the rats of group C, table 1. The latter group was deficient in choline, but not cystine, and their deaths were also accelerated by thyroid feeding, but their livers did not show the necrosis or fibrosis of choline deficiency. Thus, if the histological picture of the livers may be taken as a criterion, thyroid feeding accelerates the physiological events of cystine deficiency but not those of choline

TABLE 2

Thyroid activity and hepatic damage in cystine-deficient rats.

GROUP	DIETARY SUPPLEMENTS	NO. RATS	NO. RATS DIED	SURVIVAL TIME[1]	WEIGHT CHANGE	LIVER				THYROID
						Number examined	Fat	Necrosis	Fibrosis	
				days	*gm*					
A	Basal 2	11	11	53	−16	8	±	±	0	Hyperplasia ±
B	Basal 1	17	17	70	−18	17	+	3+	0	Hyperplasia 2+
E	Choline 0.6%	14	14	72	−13	14	0	2+	0	..
F	Choline + thyroid 0.2%	12	12	44	−19	7	0	3+	0	Normal
G	Choline + thiouracil 0.25%	8	8			8	0	0	0	..
C	Thyroid 0.2%	12	12	40	−18	5	±	4+	0	Normal
D	Thiouracil 0.25%	8	8			8	+	0	0	..

[1] Dead rats only.

deficiency. The inclusion of thiouracil in the diet, with or without the addition of choline, prevented the development of cystine deficiency necrosis (group D and G).

Dietary factors and experimental hyperthyroidism

From the data in tables 1 and 2, it was clear that a diminished level of thyroid activity, induced by thiouracil, p-aminobenzoic acid or thyroidectomy, offered protection against the deleterious effects of choline and cystine deficiency. Hyperthyroidism, induced by thyroid feeding, accelerated the death of both choline- and cystine-deficient animals, but it was not clear whether the 2 phenomena, i.e., choline deficiency (or cystine deficiency) and hyperthyroidism, actually were interrelated or whether they operated quite independently. It was thought worthwhile to determine what pertinent dietary conditions were most and least suitable for producing death due to hyperthyroidism. The diets employed are shown in table 3, and the results are summarized in table 4. The experiment was terminated after 30 days.

The incorporation of 0.4% thyroid powder into chow, or chow to which 0.8% choline had been added was quite innocuous. However, when the thyroid dosage was doubled, about one-fourth of the rats died before the termination of the experimental period. When thyroid powder was added to a good synthetic diet (3), again no rats died within 30 days. In this case, doubling the thyroid dosage resulted in the death of three-fourths of the animals. The addition of choline, as in diet 4, or to a lesser degree, methionine (diet 5) to the same good ration markedly increased the susceptibility of rats to the effects of hyperthyroidism, at both levels of thyroid feeding. This effect of choline was not as marked when choline was added to a high protein, high fat diet (diets 6 and 7) in contrast to the basal high protein, low fat diet of groups 3, 4 and 5. In contrast to groups 3 and 6, when a low protein, high fat ration deficient in choline was employed, even the lower level of thyroid feeding was fatal for all the rats in the group (8) and

TABLE 3

Composition of diets used in study of hyperthyroidism.

MATERIALS [1]	DIET NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
Chow [2]	100	99	..	..	..	..	..	..	..	..	..	..
Casein	..	..	30	30	30	30	30	5	5	5	5	5
Starch	..	..	50	49	49	30	29	60	59	59	60	59
Cotton oil	..	..	10	10	10	10	10	10	10	10	10	10
Lard	..	..	..	..	..	20	20	15	15	15	15	15
Salts [3]	..	..	4	4	4	4	4	4	4	4	4	4
Cod liver oil	..	.	5	5	5	5	5	5	5	5	5	5
Cystine	.	..	..	..	..	..	..	0.5	0.5	0.5	..	..
Cholesterol	..	..	..	..	..	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline	..	0.8	..	0.8	..	..	0.8	..	0.8	..	..	0.8
Methionine	.	..	..	..	1	..	..	..	..	1	..	..

[1] In addition all diets, except 1 and 2, contained the same vitamin supplement used with basal ration 1 (see text), but in doubled quantity.

[2] Purina Checkers, Ralston Co., St. Louis, Mo.

[3] Hubbell et al. ('37).

TABLE 4

Effect of diet on mortality due to hyperthyroidism.

DIET	0.4% THYROID			0.8% THYROID		
	No. of rats		Mean survival time [1]	No. of rats		Mean survival time [1]
	Total	Dead		Total	Dead	
			days			*days*
1	8	0	..	8	2	28
2	8	0	..	8	3	12
3	8	0	..	8	6	19
4	16	15	17	8	8	12
5	12	6	27	8	7	17
6	12	1	11	12	3	23
7	12	6	16	12	9	19
8	12	12	20	12	11	14
9	12	5	25	12	8	23
10	12	3	18	12	8	17
11	8	8	11	8	8	9
12	12	6	17	12	11	17

[1] Dead rats only.

choline partially protected against this (group 9) as did methionine (group 10). On a low protein, high fat ration deficient in cystine as well as choline (group 11), rats were more susceptible to hyperthyroidism than on any other ration studied, while the addition of choline to the diet appeared to offer some protection against both levels of thyroid feeding (group 12).

From these data it may be said that rats eating a commercial chow were less susceptible to experimental hyperthyroidism under these conditions than on any synthetic ration employed. On the whole, they were more resistant when eating high protein rather than low protein rations. No explanation can be offered for the increased susceptibility to hyperthyroidism which resulted from the addition of choline or methionine to an already adequate synthetic ration. On low protein diets, both choline and cystine deficiency markedly increased susceptibility to thyrotoxicosis, while animals deficient in both choline and cystine succumbed more rapidly than any other group in this study.

The influence of preëxisting choline deficiency upon experimental hyperthyroidism

One further experiment, summarized in table 5, was performed whose results are pertinent to this discussion. All rats were grown to 50 gm, housed in individual cages, and fed the cirrhosis-inducing basal ration 1 (described above) for 2 weeks. A representative group was sacrificed at this time and found to have a mean liver fat content of 24.3%, wet weight, range 17.1–29.8%. The remaining animals were subdivided into 4 groups. Group A remained on the same basal diet; group B was given the same diet plus 0.3% thyroid powder; group C received basal ration plus 0.3% thyroid powder and 0.8% choline. Group D was given basal ration plus 1% choline for 1 week and then offered the diet of group C. The entire experiment was then continued for 40 days. The results are summarized in table 5.

The results of such an experiment could not have been clear-cut since animals began to die of choline deficiency after

40 days. Nevertheless, the data certainly suggest that a preëxisting fatty liver affords some protection against the toxicity of thyroid feeding. While at first this would appear to be a contradiction of the findings shown in table 4 wherein choline modified the acute effects of thyroid feeding in animals on a

TABLE 5

The effect of a preëxisting fatty liver on mortality due to hyperthyroidism.

GROUP	SUPPLEMENTS TO DIET	NUMBER OF RATS		MEAN SURVIVAL TIME [1]
		Total	Dead	
				days
A	None	10	2	36
B	0.3% Thyroid	10	3	32
C	0.3% Thyroid 0.8% Choline	10	9	16
D	After 7 days on basal + 1% choline transferred to diet of group C	10	10	20

[1] Dead rats only.

All animals were fed basal ration 1 for 2 weeks in advance. At this time a representative group was found to have a mean liver fat content of 24.3%. The animals were then divided into 4 groups and continued on basal ration 1 but with supplements as indicated above.

low protein, high fat regime, it should be realized that at such levels of thyroid feeding the control group in that experiment (group 8, table 4) never developed fatty livers despite the choline deficiency. The data in table 5 do not suggest that choline deficiency, *per se,* protects against thyroid toxicity, but rather that a *preëxisting* fatty liver is less susceptible to thyroid toxicity than an otherwise normal liver.

DISCUSSION

The data which have been presented leave little doubt but that the physiological sequelae of choline and/or cystine defici-

ency are, in a considerable measure, determined by the level of thyroid activity. Hyperthyroidism, induced by feeding desiccated thyroid powder, definitely shortened the lives of rats on every diet used in this study. Cystine-deficient, choline-fed rats lived almost twice as long on the control diet as animals fed the same ration supplemented with thyroid powder. In the livers of both groups was found the acute massive necrosis previously described in cystine deficiency. In contrast, while choline-deficient, cystine-fed rats developed a focal or diffuse hepatic necrosis and fibrosis, the addition of thyroid powder to such a diet accelerated the demise of the experimental subjects, but their livers showed no specific change.

Since thyroid feeding accelerated the metabolic and histologic events of cystine deficiency it is not surprising that hypothyroidism (thiouracil feeding) delayed death due to cystine deficiency, and protected the liver cells. On the other hand, while hyperthyroidism cannot be stated to have definitely accelerated the metabolic events of choline deficiency, the various procedures calculated to induce hypothyroidism (thyroidectomy, thiouracil or p-aminobenzoic acid feeding) definitely protected rats against the hepatic necrosis and fibrosis of choline deficiency, at least for the experimental period employed in these studies. It is of interest that, while these livers showed little or no necrosis or fibrosis, they were, histologically, exceedingly fatty. Moreover, this could not have been due to a delayed accumulation of fat since hypothyroidism, even in experiments of but 21 days' duration, actually enhances the deposition of liver fat in choline-deficient rats (Handler, '47, '48). This, then, represents another instance in which the existence of a chronically fatty liver did not result in hepatic necrosis and fibrosis. That this may occur under other circumstances (nicotinamide feeding on a relatively high casein diet) has already been reported (Handler and Dubin, '46). The hepatic necrosis of choline deficiency, as usually seen, is therefore dependent upon at least a normal level of thyroid activity as well as upon the chronic fatty liver.

The experiment in which we attempted to determine the dietary conditions most propitious for death due to hyperthyroidism requires comment. No explanation is at hand to account for the relative resistance of the rats eating chow as compared to those eating our best synthetic ration. Whether this is due to the presence of some factor or factors not included in our rations, or to a different quantitative rather than qualitative composition of the known dietary factors, cannot be stated and remains a problem of potential significance in the management of hyperthyroid patients. From the results of Ershoff ('47) it appears likely that the chow used in this study may have contained the same factor, present in liver, which increases the resistance of rats to the effects of thyroid feeding. The increased susceptibility to hyperthyroidism which resulted from the addition of choline or methionine to a high protein ration, in contrast to the protection seemingly afforded when these were added to a low protein ration, also remains a confusing problem. However, it can definitely be stated that rats on low protein rations succumb to hyperthyroidism more rapidly than animals eating a high protein ration. Further protection is afforded if the high protein ration is also high in fat content rather than in carbohydrate although cystine deficiency, like choline deficiency, is more severe on high fat than low fat diets.

In contrast to clinical hyperthyroidism, simple experimental hyperthyroidism has not been found to be associated with liver damage (McIver, '42). However, 3 situations have already been described in the literature which may be said to predispose the liver to hepatic damage when the animals are also rendered hyperthyroid. Hyperthyroid, anoxic rats do show liver damage (McIver and Winter, '43); preëxisting liver damage (chloroform poisoning) is intensified by hyperthyroidism (McIver and Winter, '40); and hyperthyroid rabbits infected with either S. aureus or Shope papilloma virus (Seasly, '41, '42) also develop a hepatic necrosis. To this list, then, should be added 1 further possibility, i.e., improper diet. While, even now, it would be difficult to define the precise

dietary conditions necessary, it is obvious that a low protein ration, relatively deficient in cystine and choline, or their common precursor, methionine, may well be involved as well as a diet poor in the protective material found in commercial mixed rations and in liver. However, from the data presented herein, and from the fact that a relatively high protein diet protects the liver from the usual deleterious effects of fatty infiltration, it seems likely that hepatic damage due to hyperthyroidism is consequent upon an accelerated protein rather than carbohydrate metabolism. It remains possible, of course, that the acceleration of protein metabolism is a secondary effect of the hyperthyroidism and may be explained entirely by an accelerated demand for glyconeogenesis because of the increased rate of caloric expenditure. In summary, while simply hyperthyroidism may kill experimental animals without inducing frank hepatic damage, dietary imbalance is one of a number of predisposing influences which, when accompanied by hyperthyroidism, will lead to liver damage and death.

We have no explanation for the ameliorative action of sulfasuxidine, taurine and inositol against the hepatic necrosis and fibrosis of choline deficiency. None of these produced morphological thyroid hyperplasia, so their behavior should be differentiated from that of thiouracil and p-aminobenzoic acid. The sulfasuxidine may have acted in an indirect fashion by modifying the intestinal flora. Inositol did not operate *via* its lipotropic activity, as the livers of this group were as fatty as those of the others. It may be of some significance, however, that normal thyroid tissue contains a large concentration of inositol (Meyer, '46). The action of taurine remains entirely unexplained. Admittedly, however, these findings jeopardize our interpretation of the experiments employing thiouracil and p-aminobenzoic acid. While the latter agents definitely exerted an anti-thyroid action and the animals fed these compounds may justifiably be considered to have been in a relatively hypothyroid state, it may not be valid to attribute their beneficial effects to this aspect of their behavior. Until a rational explanation of the results obtained with

taurine, sulfasuxidine and inositol is available, it will remain possible that the results of thiouracil and p-aminobenzoic acid feeding, described herein, are non-specific. The chief justification for considering the results obtained in the present study as being due to the anti-thyroid action of these 2 compounds lies in the similar behavior of thyroidectomized rats.

The observation of thyroid hyperplasia in some of the choline-deficient as well as in certain of the choline- and cystine-deficient animals also requires some comment. Since this was not consistent, and did not occur at all in many of the animals, it would be hazardous to place much emphasis on it. It may be this variation in thyroid activity which underlies the inconsistency of the other morphological findings in choline deficiency. Further, it is not possible to state whether this thyroid hyperplasia is a direct result of a choline deficiency or whether it is secondary to events in the choline-deficient liver. In view of the readiness with which the thyroid responds to so many stimuli by morphologic hyperplasia, it would seem wise to reserve further comment until more pointed and extensive observations have been made.

SUMMARY

1. A decreased level of thyroid activity, induced by thyroidectomy, thiouracil or p-aminobenzoic acid feeding, prevents or retards the development of hepatic necrosis or fibrosis associated with choline and cystine deficiencies.

2. Although thyroid feeding hastened the death of choline-deficient rats, their livers contained little fat and showed no necrosis or fibrosis.

3. Thyroid feeding hastened the death of cystine-deficient rats; their livers exhibited the acute necrosis characteristic of this deficiency.

4. Rats living on a commercial chow were more resistant to the toxic effects of hyperthyroidism than animals on our best synthetic ration. The further addition of choline or methionine to this synthetic diet somewhat increased the susceptibility of rats to thyroid toxicity.

5. Animals on low protein diets were more susceptible to thyroid toxicity than those on high protein diets, and animals on high fat diets were somewhat more resistant than those on low fat diets. Animals deficient in both cystine and choline were more susceptible to hyperthyroidism than any other group studied.

6. The existence of an already established fatty liver appeared to protect the rat against thyrotoxicosis.

7. The inclusion of sulfasuxidine, taurine or inositol in a choline-deficient diet prevented the development of cirrhosis during the experimental period but not by virtue of lipotropic action or antithyroid activity, as judged by liver fat content and the histological appearance of the thyroid.

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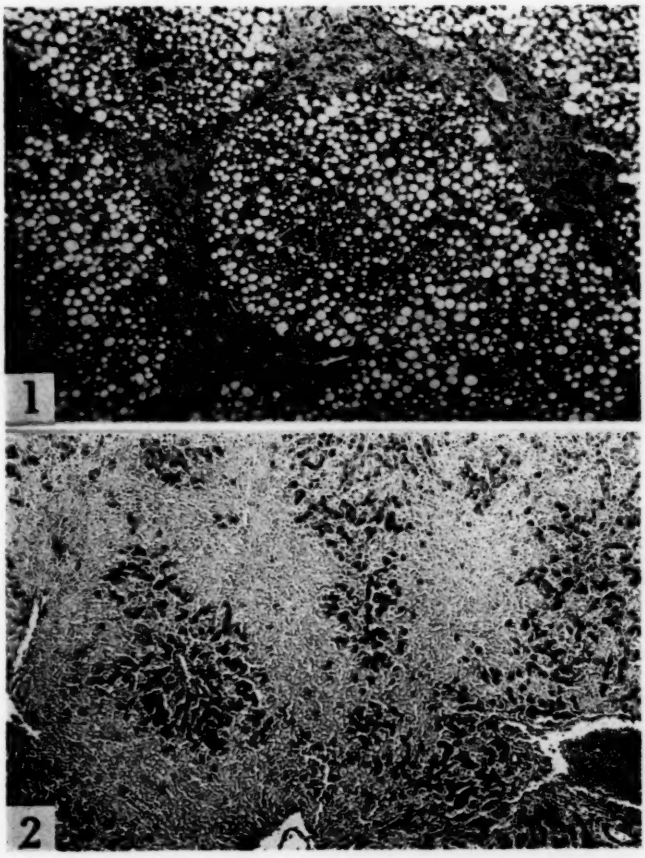

1 Photomicrograph of liver from rat on basal choline-deficient diet. Note extreme fatty infiltration of liver cells which are present. Note also relatively large areas of scarring which replace hepatic parenchyma. Such scarred areas contain vacuoles of fat as well as refractile ceroid. These areas of necrosis and fibrosis tend to involve rather large areas. Typical nodular cirrhosis was not observed in these experiments.

2 Photomicrograph of liver from rat on basal cystine-deficient diet. Note absence of fatty infiltration of the few liver cells which remain. The majority of the hepatic cells are necrotic; only here and there a few viable looking cells can be seen. There is no scarring and very little cellular reaction.

THE LYSINE, METHIONINE AND THREONINE CONTENT OF MEATS[1]

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The microbiological estimation of amino acids in meats was initiated in this laboratory by Schweigert, McIntire, Elvehjem and Strong ('44), and the contents of leucine, isoleucine and valine (Schweigert et al., '45), tryptophane (Greenhut et al., '46a, '47), threonine (Greenhut et al., '46b), and phenylalanine (Greenhut et al., '47) have been previously reported. The findings in this earlier work had indicated that meat proteins were strikingly similar with respect to their content of these 6 amino acids.

The methods employed in the previous work have been extended to the determination of methionine and lysine in meats, and to the assay of a greater variety of meat samples for their threonine content than were studied in the earlier work. In the present study *Leuconostoc mesenteroides P-60* was used for the determination of methionine and lysine, while *Streptococcus faecalis R* was employed in the methods for methionine and threonine. The data obtained for the lysine, methionine and threonine content of meats have been subjected to a statistical analysis in an attempt to evaluate differences between cuts and the variation encountered in repeated analyses.

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EXPERIMENTAL

Preparation of samples

Fresh cuts of beef, lamb, pork, veal and whole beef organs (table 2) were obtained at a local packing house, and were immediately prepared for analysis. Two of the cuts from each of the species represented were divided into uniform halves, and 1 portion from each of these was roasted according to methods described by McIntire et al. ('43). After the meats had been trimmed to remove bone, gristle and excess fat, they were passed through an electric food chopper 4 to 6 times and were mixed thoroughly to give homogeneous preparations. To insure the same moisture content in samples taken for nitrogen analysis and for hydrolysis, weighings were made simultaneously. Nitrogen determinations were carried out by a macro-Kjeldahl procedure. Hydrolysates were prepared by autoclaving 10-gm samples with 200 ml 2N HCl for 5 hours at 15 lbs. pressure in covered Erlenmeyer flasks. Preliminary studies had shown that increases in the concentration of the acid or the length of time of hydrolysis did not result in higher values for these 3 amino acids. The hydrolysates were filtered at room temperature, diluted to a convenient volume and stored at 5°C. Prior to each assay, portions of the hydrolysates were neutralized and further diluted to the appropriate concentrations required for each amino acid.

Assay procedures

The microbiological assay techniques described by Schweigert et al. ('44) have been applied with some modification to the determinations of lysine, methionine and threonine in fresh and cooked meats. To permit the simultaneous use of different organisms for the assay of several amino acids with 1 basal medium, the uniform medium developed by Henderson and Snell ('48) was adopted with respect to all constituents except for the levels of certain amino acids. These amino acids were incorporated at the levels which had been routinely

TABLE 1

Basal medium for assays of lysine, methionine and threonine.[1]

CONSTITUENT	AMOUNT/ 100 ML, FINAL CONCENTRATION	CONSTITUENT	AMOUNT/ 100 ML, FINAL CONCENTRATION
Glucose (*gm*)	2.0	*Amino acids*	
Na acetate (*gm*)	0.1	DL-alanine (*mg*)	20
Na citrate (*gm*)	2.0	L-arginine · HCl (*mg*)	10
NH_4Cl (*gm*)	0.3	DL-aspartic acid (*mg*)	40
K_2HPO_4 (*gm*)	0.5	L-cystine (*mg*)	20
Salts C		L-glutamic acid (*mg*)	40
$MgSO_4 \cdot 7H_2O$ (*mg*)	80	Glycine (*mg*)	10
NaCl (*mg*)	4	L-histidine · HCl · H_2O (*mg*)	10
$FeSO_4 \cdot 7H_2O$ (*mg*)	4	DL-isoleucine (*mg*)	20
$MnSO_4 \cdot 7H_2O$ (*mg*)	16	L-leucine[2] (*mg*)	10
Adenine sulphate (*mg*)	1	L-lysine · HCl · H_2O (*mg*)	20
Guanine hydrochloride (*mg*)	1	DL-methionine (*mg*)	20
Uracil (*mg*)	1	DL-phenylalanine (*mg*)	10
Xanthine (*mg*)	1	L-proline (*mg*)	5
Thiamine (*μg*)	100	DL-serine (*mg*)	20
Riboflavin (*μg*)	100	DL-threonine (*mg*)	20
Pyridoxal (*μg*)	20	DL-tryptophane (*mg*)	10
Ca pantothenate (*μg*)	100	L-tyrosine (*mg*)	10
Niacin (*μg*)	100	DL-valine (*mg*)	20
p-Aminobenzoic acid (*μg*)	20		
Biotin (*μg*)	1		
Folic acid (*μg*)	1		

[1] Appropriate amino acid omitted from the medium.

[2] DL-leucine substituted at 20 mg level for the methionine assays to avoid possible methionine contamination. (Riesen et al., '46.)

used in prior work, and the constituents of the medium as used are presented in table 1.

The assays were conducted on a semi-micro scale in which the final volume of medium per tube was 2 ml. In all cases the standard curves were based on at least 5 tubes at each of 6 different levels. The standard solutions were prepared to contain 18 μg of DL-threonine, 8 μg of L-methionine,[2] and 25 μg of L-lysine (added as the monohydrochloride). Samples were

[1] Kindly supplied by Dr. R. T. Major, of Merck and Co., Rahway, New Jersey.

TABLE 2

Analysis of meats.

CUT OF MEAT	PROTEIN	LYSINE				METHIONINE				THREONINE			
		No. of assays	In meat [1]	In protein [2]	Retention	No. of assays	In meat	In protein	Retention	No. of assays	In meat	In protein	Retention
	%		%	%	%		%	%	%		%	%	%
Veal													
Shank	21.7	8	1.73	8.0		5	0.43	2.0		3	0.95	4.4	
Shoulder	20.4	8	1.63	8.0		5	0.47	2.3		4	0.86	4.2	
Roast shoulder	27.4	6	2.09	8.0	97	6	0.63	2.3	102	5	1.15	4.2	101
Loin	17.1	6	1.48	8.3		7	0.39	2.3		4	0.77	4.5	
Round	20.4	7	1.63	8.0		5	0.45	2.2		6	0.86	4.2	
Roast round	29.0	4	2.52	8.7	114	5	0.67	2.3	109	4	1.30	4.5	111
Group mean				8.1				2.3				4.3	
Lamb													
Shank	11.7	6	0.96	8.2		7	0.28	2.4		5	0.53	4.5	
Roast shank	12.5	5	1.05	8.4	102	7	0.27	2.2	90	5	0.55	4.4	96
Shoulder	15.8	6	1.26	8.0		6	0.38	2.4		4	0.71	4.5	
Loin	13.0	4	0.99	7.6		4	0.30	2.3		3	0.56	4.3	
Leg	16.5	4	1.37	8.3		6	0.38	2.3		5	0.69	4.2	
Roast leg	27.5	5	2.17	7.9	96	5	0.60	2.2	92	4	1.10	4.0	92
Group mean				8.1				2.3				4.3	
Pork													
Picnic ham	16.5	6	1.32	8.0		6	0.36	2.2		7	0.69	4.2	
Butt	12.2	8	0.96	7.9		6	0.27	2.2		2	0.54	4.4	
Roast butt	22.8	6	1.94	8.5	108	7	0.50	2.2	99	6	1.00	4.4	100
Loin	17.4	3	1.38	7.9		8	0.40	2.3		5	0.76	4.4	
Ham	13.9	6	1.15	8.3		4	0.35	2.5		3	0.64	4.6	
Roast ham	27.6	2	2.10	7.6	91	5	0.64	2.3	91	3	1.27	4.6	98
Group mean				8.1				2.2				4.4	
Beef													
Chuck	17.4	6	1.46	8.4		4	0.40	2.3		7	0.77	4.4	
Roast chuck	22.7	5	1.84	8.1	99	5	0.50	2.2	98	6	0.95	4.2	97
Rib	16.7	3	1.32	7.9		4	0.37	2.2		5	0.73	4.4	

Roast chuck	22.7	5	1.84	8.1	99	5	0.50	2.2	98	6	0.95	4.2	97
Rib	16.7	3	1.32	7.9		4	0.37	2.2		5	0.73	4.4	

TABLE 2 *(continued)*

Analysis of meats.

CUT OF MEAT	PROTEIN	LYSINE				METHIONINE				THREONINE			
		No. of assays	In meat [1]	In protein [2]	Retention	No. of assays	In meat	In protein	Retention	No. of assays	In meat	In protein	Retention
	%		%	%	%		%	%	%		%	%	%
Beef													
Loin end	17.6	4	1.35	7.7		5	0.39	2.2		4	0.74	4.2	
Round	20.6	4	1.44	7.0		4	0.45	2.2		4	0.89	4.3	
Roast round	33.4	3	2.94	8.8		3	0.80	2.4	114	4	1.47	4.4	106
Group mean				8.0				2.2				4.3	
Brain	10.6	5	0.69	6.5		3	0.22	1.9		7	0.46	4.3	
Brain	10.8	5	0.65	6.0		4	0.19	1.8		8	0.45	4.2	
Brain	10.3	3	0.71	6.9		4	0.19	1.8		4	0.46	4.5	
Mean				6.4				1.8				4.3	
Heart	17.4	2	1.35	7.8		7	0.37	2.1		8	0.77	4.4	
Heart	17.7	5	1.36	7.7		3	0.37	2.1		7	0.80	4.5	
Mean				7.8				2.1				4.5	
Kidney	16.7	5	1.13	6.8		3	0.28	1.7		6	0.73	4.4	
Kidney	16.4	3	1.15	7.0		6	0.33	2.0		8	0.74	4.5	
Kidney	16.0	5	1.07	6.7		6	0.29	1.8		3	0.69	4.3	
Mean				6.8				1.9				4.4	
Liver	21.0	5	1.47	7.0		3	0.50	2.4		6	0.88	4.2	
Liver	20.8	4	1.45	7.0		3	0.48	2.3		6	0.90	4.3	
Liver	20.4	4	1.43	7.0		4	0.45	2.2		3	0.90	4.4	
Mean				7.0				2.3				4.3	
Tongue	20.5	4	1.58	7.7		7	0.41	2.0		6	0.84	4.1	
Tongue	20.2	6	1.53	7.5		6	0.40	2.0		7	0.89	4.4	
Mean				7.6				2.0				4.3	
Standard deviation				0.54				0.21				0.26	
Coefficient of variability				7.0				9.6				6.0	

[1] Per cent amino acid as used throughout signifies grams of amino acid per 100 gm of meat (or meat protein).

[2] Calculated to 16% nitrogen.

added in duplicate at each of 3 different levels, with all necessary measurements being made with either a Cannon dispenser [3] or the Cannon-Riesen aliquoter.[4] Assay tubes were sterilized by autoclaving at 15 lbs. for 15 minutes, and were inoculated with 1 drop per tube of a 25 ml sterile saline suspension of the test organism, centrifuged from 5 ml enriched medium in which growth had proceeded for 24 hours. The tubes were incubated at 37°C. for 72 hours, and the acid produced was titrated with the aid of a quinhydrone pontentiometric titrator.[4]

RESULTS AND DISCUSSION

The results of repeated assays for lysine, methionine and threonine in the various cuts of meats are presented in table 2. The amino acid content is expressed as the per cent found in the meat (grams amino acid per 100 gm of tissue) and as the per cent in the protein (calculated to 16% nitrogen). In the case of the cooked samples, the retention of these amino acids has been calculated on the basis of the total amount of each found in the whole cut before and after roasting. The number of assays on which the mean values are based is also indicated.

All data were treated statistically by the analysis of variance technique, as shown in table 3. A combined analysis of the data for each amino acid was used, since it was found that the mean squares within samples were homogeneous for each of the 3 amino acids. The F values obtained by this analysis indicate that there are significant differences in the lysine and methionine contents of the various meat samples, but no significant differences are found in the threonine contents. The specific location of these significant differences has been ascertained by means of the *t* test.

[3] To be published by M. D. Cannon.

[4] A complete description of the equipment being used in this laboratory for the preparation of microbiological assays will be published in a later paper.

TABLE 3

Analysis of variance for data obtained in repeated assays for lysine, methionine and threonine in meats.

SOURCE OF VARIATION	LYSINE			METHIONINE			THREONINE		
	Degrees of freedom	Mean square	F value	Degrees of freedom	Mean square	F value	Degrees of freedom	Mean square	F value
Between all samples	36	2.0178	6.84 [1]	36	0.1520	3.49 [1]	36	0.0860	1.25
Between cuts within veal	5	0.3977	1.35	5	0.0559	1.29	5	0.0733	1.06
Between cuts within lamb	5	0.3977	1.36	5	0.0286	< 1.00	5	0.1246	1.81
Between cuts within pork	5	0.4236	1.44	5	0.0669	1.54	5	0.0852	1.24
Between cuts within beef	5	1.3715	4.65 [1]	5	0.0160	< 1.00	5	0.0506	< 1.00
Between cuts within organs	8	0.2414	< 1.00	8	0.0324	< 1.00	8	0.1027	1.49
Between species for muscle tissues	3	0.0743	< 1.00	3	0.0399	< 1.00	3	0.0289	< 1.00
Between organs	4	3.2150	10.90 [1]	4	0.3675	8.45 [1]	4	0.1299	1.89
Between muscle tissues and organs	1	44.6630	151.45 [1]	1	2.7855	64.03 [1]	1	0.0002	< 1.00
Within samples	144	0.2949	...	151	0.0435	...	150	0.0689	...

[1] Indicates that F value exceeds 1% level of significance.

Lysine

Application of the *t* test to the lysine data indicates that the mean values of 6.4% in brain, 6.8% in kidney and 7.0% in liver are significantly lower than the mean of 8.0% lysine found in the protein of the beef muscle tissues, when considered at a 1% level of significance. Extending this test further, it can be shown that the mean for beef tongue is significantly lower than the group means for veal, lamb and pork muscle tissues, while the mean of 7.8% lysine in heart proteins is no different from that of the other muscle tissues. No such differences can be detected within the cuts of the various muscle tissues, except in the case of round and roast round of beef. This latter difference appears to be significant, but since the values are based on only 1 preparation of hydrolysate and on only 4 and 3 assays, respectively, it is felt that no practical significance can be attached to the difference. However, it is possible that the difference may be peculiar to this particular sample of meat or to the preparation thereof.

Microbiological methods for the determination of lysine have been reported by many workers (Dunn et al., '44; Hier et al., '45; Stokes et al., '45; Guirard et al., '46), but data regarding the lysine content of meats and meat proteins are limited. Hier et al. ('45) reported 7.9% in beef muscle protein, and Stokes et al. ('45) found 6.1% in beef liver, both workers having used *S. faecalis* as the assay organism. These values compare favorably with the 8.0% lysine in beef muscle tissues and the 7.0% in beef liver found in the present work by use of the same organism. Beach et al. ('43), employing a chemical procedure for the analysis of lysine, found 8.11, 9.62, 8.68 and 8.65% in the muscle tissue proteins of beef, veal, lamb and pork, and 7.10, 6.02, 6.21 and 5.98% in beef heart, liver, kidney and brain, respectively. These workers pointed out that one of the outstanding differences between organ and muscle tissues found in their analyses of 10 different amino acids was the lower level of lysine in organs. The present work serves to give statistical verification to this difference,

though the average differences appear to be considerably smaller than those found by Beach et al.

Methionine

The data in table 2 show that the proteins of the muscle tissues and of beef liver average 2.2 to 2.3% in their content of methionine, with no significant differences discernible among any of these groups. Values obtained for beef brain, kidney and tongue are 1.8, 1.9 and 2.0%, all of which are significantly lower than those for the muscle tissue groups. The 2.1% value found for beef heart appears to be significantly lower than the 2.3% found in veal and lamb muscle tissues, but is higher than the 1.8 and 1.9% methionine found in beef brain and kidney proteins.

Earlier work reported from this laboratory by Riesen et al. ('46) indicated a considerably higher content of methionine in some of the meat tissues. Three different organisms, *L. arabinosus, Leuc. mesenteroides* and *S. faecalis,* gave essentially the same values under the earlier conditions, and some of the values reported included 3.03% methionine in the proteins of pork muscle, 3.09% in beef heart, 1.93% in beef kidney and 2.55% in beef brain. In the present study, both *S. faecalis* and *Leuc. mesenteroides* were used interchangeably with excellent agreement, though all results were consistently lower than previously reported.

A comprehensive study of the methionine content of meats has been conducted by Lyman et al. ('46a), who reported values ranging from 2.09% in beef kidney protein to 2.50% in the protein of beef round. Though in general their values are somewhat higher than those found in this present study, essentially the same relative differences were found between muscle tissues and organs, with the exception of beef liver, which they found to be almost as low in methionine as some of the other organs, e.g., beef tongue. Other values found in the literature for the methionine content of meats include 2.0% in beef liver (Stokes et al., '45), 2.75% in beef muscle

(Riesen et al., '46), 3.06 to 3.62% in various muscle tissues (Beach et al., '43) and 4.12% in beef round (Edwards et al., '46). The 2 former groups of workers used a microbiological technique; the latter groups employed chemical methods in their work. In general, values obtained by chemical methods appear to be considerably higher than those found by microbiological means, though Lyman et al. ('46b) were able to obtain excellent agreement between 2 different microbiological methods and a colorimetric method.

Threonine

Although the proteins of various meat muscle tissues and organs show significant differences in lysine and methionine content, they are remarkably similar in threonine content. No statistically significant difference could be detected among any of the various groups of meats, with individual cuts and organs averaging 4.0 to 4.6% in the proteins, giving an over-all average of 4.3%.

In a previous publication by Greenhut et al. ('46b) from this laboratory on the determination of threonine in natural products with *S. faecalis,* values were reported on several different meat tissues. A rather high degree of variation had been encountered, and values ranged from 3.7 to 4.7% threonine in the proteins. Some of this variation may be attributed to the fact that the assays had been conducted with samples which had been stored at — 5°C. for 2 to 3 years, with brief partial thawing at intervals of 3 to 4 months. These conditions may have been responsible for destruction of some threonine, or may have led to nitrogen losses which would account for high values obtained in calculating to a 16% nitrogen basis. In general, however, the present results are in good agreement with the earlier work.

Other workers have reported 5.4% threonine in beef muscle proteins (Hier et al., '45), 3.8% in beef liver proteins (Stokes et al., '45) and 3.34 to 3.92% in various muscle tissues and beef organs (Beach et al., '43).

Standard deviation

The standard deviations for lysine, methionine and threonine, as shown in table 2, are 0.54, 0.21 and 0.26, respectively, with reference to the per cent of amino acid found in the meat proteins. The coefficient of variability, which expresses the standard deviation in per cent of the arithmetic mean, was 7.0% for lysine, 9.6% for methionine and 6.0% for threonine. Both of these statistical entities are measures of the random, uncontrolled variation found in repeated assays, and as such serve as a guide to the degree of confidence that can be placed on values determined from a limited number of assays.

The values presented in table 2 for the retention of these amino acids indicate that there is no significant reduction in their total amounts during the cooking process. There is, however, a wide range in the amount of protein found in the different meats. Pork and lamb cuts tend to have less protein than the beef and veal, reflecting a higher content of fat in the former. From a nutritional standpoint, these wide differences in protein content are of importance in the evaluation of total amino acid intake, even though the meat proteins themselves appear to be quite similar.

SUMMARY

Leuconostoc mesenteroides was used for the determination of lysine and methionine in meats. *Streptococcus faecalis* was used for similar determinations of methionine and threonine. The analyses were made on acid hydrolysates of the crude homogeneous meat samples. The average threonine content of 24 different skeletal muscle tissues was 4.4% of the crude protein, while the lysine content was 8.1% and the methionine content was 2.3%. A statistical analysis indicated that no significant differences existed between the results obtained for the muscle tissue proteins, but that beef brain, kidney and tongue were significantly lower in methionine while beef brain, kidney and liver were significantly lower in lysine. Studies on

retention show that these amino acids are not lost or destroyed in meats during the cooking process.

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RELATIVE SIGNIFICANCE OF GROWTH AND METABOLIC RATE UPON THE UTILIZATION OF VITAMIN A BY THE RAT [1]

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Recent experiments indicate the importance of thyroxine in the conversion of carotene to vitamin A in the rat (Johnson and Baumann, '47b), but it is not entirely clear whether the requirement for the vitamin itself increases with increased metabolic rate. Claims to the latter effect have been frequent (Abelin, '33; v. Euler and Klussmann, '32; Schneider and Widmann, '34; Wendt, '35; Greaves and Schmidt, '36; Sure and Buchanan, '37), although contradictory data have also been recorded. When groups of normal and hyperthyroid rats containing uniform stores of vitamin A were fed a diet low in vitamin A, the amounts of this factor remaining in the liver were usually at least as high in the hyperthyroid group as in the controls (Logaras and Drummond, '38; Baumann and Moore, '39), and in some series the administration of thyroxine actually appeared to decrease the rate of utilization of vitamin A. Hyperthyroid rats fed moderate amounts of fish liver oil stored approximately as much vitamin A as control rats

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[2] National Institute of Health Junior Research Fellow.

(Johnson and Baumann, '47b). However, factors that alter the metabolic rate usually also alter the rate of growth, and a large animal might reasonably be expected to require more vitamin than a small one. The present experiments were therefore designed to evaluate the relative significance of the 2 factors, growth and metabolic rate, upon the metabolism of vitamin A in the rat.

METHODS

The experiments were performed on series of comparable weanling rats either from our own stock colony or from the Sprague-Dawley Company; rats from the 2 sources proved to be quite similar. The groups within each series were comparable in weight, age and sex. At the beginning of the experiment, each rat was fed 6 drops (approximately 0.12 gm) of a vitamin A concentrate prepared by mixing 1 gm of halibut liver oil (approximately 60,000 I.U. per gm) with 3 gm of cottonseed [3] oil. Three drops of the mixture were fed in the morning and the other 3 drops, 8 hours later. This was sufficient to produce hepatic stores of approximately 250 μg of vitamin A, as determined by analyses made on animals killed 72 hours after the administration of the second portion of the concentrate. The animals were then maintained on various diets low in vitamin A and subjected to the conditions indicated below in order to promote different metabolic rates or different rates of growth.

The basal diet had a percentage composition of vitamin A-free casein 18, dextrin 65, cottonseed [3] oil 5, brewers' yeast 8, and salts (Wesson, '32) 4. Sixteen drops of a propylene glycol solution [4] containing 10,000 U.S.P. units per gram of a crystalline vitamin D from ergosterol were added per 10 kg of diet. Decreased growth rates were effected either by restricting the caloric intake to ½ or ¾ of that consumed by the control animals, or by feeding a diet low in either thiamine or trypto-

[3] Wesson.

[4] Drisdol, containing 400 units of vitamin D, Winthrop Chemical Company, Inc., New York, N. Y.

phane. In the diet low in thiamine, brewers' yeast autoclaved for 4 hours at 15 pounds pressure replaced the ordinary yeast in the low A diet. As the depletion period was prolonged, enough thiamine hydrochloride was fed by dropper to enable the rats of this group to maintain weight. In the diet low in tryptophane, the casein of the basal ration was replaced by an equivalent amount of a casein hydrolysate prepared according to the method of Woolley ('46). Since this ration contained 8% of yeast, there was enough tryptophane present to permit the animals to maintain weight for 2 months with slight growth during the first few weeks. A relatively rapid rate of growth, 4 gm/rat/day during the depletion period, was produced in 1 group by the substitution of 0.5% of a solubilized whole liver preparation [5] for an equivalent amount of brewers' yeast; comparable rats on the control diet gained 3.3 gm/day.

The basal metabolic rates of the rats were decreased by feeding either thiourea incorporated into the basal diet at a level of 0.5%, or 2-thiouracil administered in the drinking water at a level of 0.1%. Increases in metabolic rates were produced by feeding desiccated thyroid at a level of 1 mg per gram of body weight per day. This material was suspended in water at a concentration that supplied the required amount in 3 drops of the suspension. It was prepared fresh every 3 days, and was kept stored in a refrigerator. In 1 experiment, 2,4-dinitrophenol was used to increase the metabolic rates in rats fed the basal low A diet. The compound was dissolved in dilute aqueous $NaHCO_3$ solution at a concentration of 4 mg per milliliter, and the solution was injected subcutaneously at a level of 40 mg per kilogram of body weight per day. The daily dose was injected in 2 equal portions, given 8 hours apart. Quantitative data on the consumption of oxygen by the rats were obtained in 1 series by means of an apparatus modified from that described by Schwabe and Griffith ('38).

After suitable intervals of time, selected to permit the vitamin A content of the livers to decrease measurably, the rats were killed by decapitation, and the livers and kidneys were

[5] Lederle liver preparation no. 1432, found to contain 1.8 μg vitamin A per gram.

removed and analyzed individually for vitamin A by the procedure described previously (Johnson and Baumann, '47a). Analyses were either made periodically, after 15, 30 and 60 days, or in the later series, after 45 days of depletion.

EXPERIMENTAL

Depletion of vitamin A reserves in growing and non-growing rats

In line with the observations of others, rats lost vitamin A from the liver when fed diets deficient in this vitamin for long periods of time. In a typical series (A, table 1) in which the basal low A diet was fed *ad libitum,* the liver stores decreased from an initial value of 228 μg at the beginning of the experiment to 42 μg 60 days later. In a second series, B, the values declined from an initial 238 μg to 41 μg after 45 days. In both series the rats grew well, an average of 208 gm in 60 days in series A, and an average of 175 gm in 45 days in series B. However, when the growth of the rats was hindered by feeding an inadequate amount of food, or by feeding diets that were deficient in tryptophane or in thiamine, the amounts of vitamin A that remained in the liver were appreciably higher than in rats that grew well. Rats fed only ½ the amount of food consumed by the control group gained only 33 gm in 60 days in series A, and 33 gm in 45 days in series B (table 1). The amounts of hepatic vitamin A remaining at the end of the experiment were 111 μg and 188 μg in the 2 series, respectively, as compared to values of 42 and 41 μg in the control groups at these times. Thus, the animals that grew well lost 2–4 times as much of the original hepatic vitamin A as did the partially starved rats. Essentially similar results were observed when the growth rate was decreased by feeding diets inadequate in tryptophane or in thiamine. The low-tryptophane rats grew only 33 gm in 60 days, and contained 91 μg of vitamin A, as compared with 42 μg found in the normally growing control animals. A diet low in thiamine resulted in a growth of 43 gm in 45 days, with 108 μg of vitamin A remaining in the liver,

as compared with 41 μg found in the control animals that gained 175 gm.

TABLE 1

The influence of body size upon the depletion of vitamin A from the liver.[1]

DIET	FEEDING PERIOD	BODY WT. GAINS	VITAMIN A			
			Liver	Kidney	Per gm of kidney	Total: kidney + liver
	days	*gm.*	*μg*	*μg*	*μg*	*μg*
Series A						
Initial stores	3	..	228	1	1.8	229 (206–261)
Basal, *ad lib.*	30	104	156	18	13.8	174 (143–216)
Basal, *ad lib.*	60	208	42	5	3.5	47 (33– 59)
Basal, ½ caloric	30	30	195	1	1.5	196 (191–229)
intake	60	33	111	1	1.1	112 (111–123)
Low tryptophane	15	25	225	1	1.3	226 (175–232)
Low tryptophane	30	36	145	1	1.7	146 (128–157)
Low tryptophane	60	33	91	1	1.2	92 (45–123)
Series B						
Initial stores	3	..	238	3	3.9	241 (197–259)
Basal, *ad lib.*	30	136	126	12	9.3	138 (109–185)
Basal, *ad lib.*	45	175	41	29	16	70 (52– 87)
Basal, ½ caloric	30	28	205	2	3.3	207 (192–232)
intake	45	33	188	2	2.1	190 (172–222)
Thyroid,[2] ½ caloric	30	—5	186	1	1.4	187 (177–214)
intake	45	5	172	2	1.6	174 (167–180)
Low thiamine	30	34	179	2	2.4	181 (138–221)
Low thiamine	45	43	108	3	3.0	111 (93–126)

[1] Each figure represents the mean of 4 animals.

[2] Thyroid fed at a level of 1 mg per gm of body wt. per day.

Effect of metabolic rate upon vitamin A retention

Thiourea and thiouracil in doses known to lower the basal metabolic rate by 20 to 35% (Reineke et al., '45; Christensen, '45), markedly delayed the disappearance of vitamin A from the liver. In series A (table 2) rats fed the low A diet plus 0.5% thiourea still retained 186 μg of hepatic vitamin A out of an original 282 μg after 60 days of depletion, as compared to

only 23 μg remaining in the control groups. Expressed as microgram of vitamin A lost from the liver, the controls lost 126 μg in 30 days, and 259 μg in 60 days, whereas those receiving thiourea lost only 20 and 96 μg at these times, respectively. Rats receiving 0.1% thiouracil in the drinking water (table 2, series B) likewise retained more hepatic vitamin A than control rats; after 45 days of depletion, the average amounts retained were 131 μg and 76 μg, respectively. Since, however, the thio-compounds had also retarded the rate of growth, e.g., the rats fed thiourea grew only 59 gm in 60 days as compared

TABLE 2

The influence of basal metabolic rate upon the depletion of vitamin A from the liver.[1]

DIET	FEEDING PERIOD	BODY WT. GAINS	VITAMIN A			
			Liver	Kidney	Per gm of kidney	Total: kidney + liver
	days	*gm*	*μg*	*μg*	*μg*	*μg*
Series A						
Initial stores	3	..	282	2	3.3	284 (260–320)
Basal, *ad lib.*	30	104	156	11	8.4	167 (129–194)
Basal, *ad lib.*	60	210	23	23	11.8	46 (30– 64)
Basal + thiourea [2]	30	38	262	0.4	0.57	262 (241–287)
Basal + thiourea [2]	60	59	186	0.4	0.64	186 (164–216)
Basal + thyroid [3]	30	59	171	3	2.0	174 (159–185)
Series B						
Initial stores	3	.	265	2	2.6	267 (234–294)
	45	171	76	8	4.7	84 (40–109)
Basal, ½ caloric intake + thyroid [3]	45	8	149	1	1.0	150 (115–212)
Basal + thyroid [3]	45	111	48	5	2.6	53 (41– 66)
Basal + thiouracil [4]	45	131	131	3	1.8	134 (121–140)

[1] Each figure represents the mean of 4 animals.
[2] Thiourea incorporated into the basal diet at a level of 0.5%.
[3] Thyroid fed at a level of 1 mg per gm of body wt. per day.
[4] 2-Thiouracil administered at a level of 0.1% in drinking water.

to a gain of 210 gm by the control groups (table 2), the observed differences in vitamin retention could not be ascribed solely to the altered metabolic rate. Indeed, the effects of the thiourea, both on the reduction in growth rate and on increased vitamin retention (table 2, series A), were of the same order of magnitude as those previously observed when the intake of food was restricted by 50% (table 1).

Nevertheless, an effect on vitamin A by the basal metabolic rate as distinct from body size or the growth process is suggested in table 2, series B. Rats fed 1 mg of thyroid per gram of body weight grew 111 gm in a depletion period of 45 days, as compared to 131 gm by a comparable group receiving thiouracil, yet in spite of this similarity in growth the rats receiving thyroid lost their reserves of vitamin A much faster than those receiving thiouracil, the amounts of vitamin remaining in the liver being 48 and 131 μg in the 2 groups, respectively.

In an attempt to determine the relative significance of growth and metabolic rates on the disappearance of vitamin A, rats with uniform stores of the vitamin were fed 3 levels of food, the amount consumed *ad libitum*, or ¾ or ½ of this amount. At each level of intake, 1 group received the basal low A diet, another the diet plus supplements of desiccated thyroid, while a third received thiouracil in the drinking water. Rats on the control diet *ad libitum* gained 148 gm in 45 days. All other groups gained lesser amounts, the least being a gain of 9 gm by the low calorie group receiving thyroid (table 3). The amounts of vitamin A present in the liver after 45 days definitely indicated the importance both of the metabolic rate and of body size for the utilization of this vitamin. Normal rats whose caloric intakes were restricted by 25% were approximately as big as full-fed rats receiving either thyroid or thiouracil, the gains in 45 days being 82, 89 and 81 gm, respectively. In spite of this similarity in size, the amounts of vitamin A retained varied inversely with the metabolic rate. The rats on thiouracil, with an O_2 consumption 26% below normal contained 229 μg of vitamin A, a loss of only 61 μg from the

TABLE 3

The relative significance of body size and basal metabolic rate on vitamin A retention after 45 days.[1]

DIET	BODY WT. GAINS	VITAMIN A			CHANGE IN O_2 CONSUMPTION
		Liver	Kidney	Total: kidney + liver	
	gm	*μg*	*μg*	*μg*	%
Initial stores	..	290	2	292 (284–305)	..
Low A, *ad lib.*	148	136	33	169 (116–197)	0
Low A, ¾ caloric intake	82	182	8	190 (170–246)	— 11
Low A, ½ caloric intake	49	187	3	190 (185–231)	+ 9
Low A + thyroid,[2] *ad lib.*	89	127	5	132 (74–175)	+ 220
Low A + thyroid,[2] ¾ caloric intake	42	190	3	193 (160–214)	..
Low A + thyroid,[2] ½ caloric intake	9	212	3	215 (133–232)	+ 238
Low A + thiouracil,[3] *ad lib.*	81	229	2	231 (187–297)	— 26
Low A + thiouracil,[3] ¾ caloric intake	83	162	3	165 (152–214)	— 25
Low A + thiouracil,[3] ½ caloric intake	42	247	2	249 (201–289)	— 25.5
Low A + Dinitrophenol[4]	125	144	5	149 (126–189)	+ 94
Solubilized liver preparation[5]	179	113	28	141 (100–193)	— 6

[1] Each figure represents the mean of 5 animals.

[2] Thyroid fed at a level of 1 mg per gm of body wt. per day.

[3] 2-Thiouracil administered at a level of 0.1% in drinking water.

[4] 2,4-Dinitrophenol given in 2 daily injections at a level of 20 mg of the drug per kg of body wt. per injection.

[5] Lederle's liver preparation no. 1432, incorporated into the basal low A diet at a level of 0.5%.

original stores of 290 μg (table 3). The normal rats on mild caloric restriction retained 182 μg of vitamin A, a loss of 108 μg, while rats of similar size fed thyroid retained only 127 μg, a loss of 163 μg. The latter rats had a metabolic rate 220% above normal.

In agreement with the results cited in the introduction, desiccated thyroid appeared to have variable effects on vita-

min A retention. In some series (table 2, series B; table 3, groups fed *ad libitum*), the hyperthyroid rats retained less vitamin A than faster-growing rats in the corresponding control groups. This result emphasizes the fact that an increased metabolic rate *per se* may increase the rate of utilization of vitamin A. In other series (table 2, series A; table 3, series receiving ½ or ¾ caloric intake) the rats receiving thyroid actually retained more vitamin A than control rats not receiving thyroid, presumably because the hyperthyroid rats grew only about ½ as well as their respective controls. Since the dose of thyroid employed was sufficient to increase the metabolic rate by 220–238%, the fact that the hyperthyroid rats contained more vitamin A than their controls leads to the conclusion that a decrease of 50% in the growth rate can have a greater quantitative significance on the retention of hepatic vitamin A than a 3-fold increase in the metabolic rate.

The administration of 2,4-dinitrophenol increased the metabolic rate by 94% without any comparable effect either on the growth rate or on the retention of vitamin A. The average growth in the group receiving dinitrophenol was 125 gm in 45 days as compared to 148 gm in the control group, and the retention of vitamin A was essentially the same in the 2 groups, 144 and 136 μg, respectively. In other words, a decrease of 23 gm in body size preserved more vitamin A than that lost because of a 94% increase in metabolic rate. The addition of a soluble liver extract to the basal low A diet resulted in a slightly decreased retention of vitamin A. Rats fed the liver extract gained an average of 179 gm in 45 days as compared to 148 gm gained by the control group. Hepatic stores of the vitamin at this time averaged 113 μg in the faster-growing group as compared to 136 μg in the controls (table 3).

Translocation of vitamin A from liver to kidney

In a previous study an increase in the vitamin A content of the kidneys was observed in rats that were rapidly losing hepatic vitamin A under the influence of dibenzanthracene

(Baumann et al., '42). The present experiments indicate that this shift of vitamin A to the kidneys is not necessarily dependent upon the presence of dibenzanthracene, but also occurs in normal rats depleted of vitamin A. An increase was observed in both the amount and the concentration of vitamin A in the kidneys of all rats that grew appreciably during the depletion period (tables 1, 2 and 3); and in a few groups (table 1, series B; table 2, series A) the amount of vitamin A in the kidney approached that in the liver as the depletion period was prolonged. However, no comparable increase in the concentration of vitamin A occurred in kidneys from rats whose growth had been restricted during the depletion period, and the amounts were particularly low in the kidneys of rats exposed to thiourea.

DISCUSSION

The need for certain of the B vitamins is generally regarded as depending directly and almost proportionally on the metabolic rate (Cowgill and Palmieri, '33; Drill, '38; Drill and Overman, '42). The present experiments indicate that the depletion of vitamin A from the liver depends upon metabolic rate to some extent, but that body size may affect vitamin A reserves to a greater extent than metabolic rate. In certain other respects, however, the retention of vitamin A in the liver seemed to parallel that of the B vitamins more closely. It has been observed that a reduction in food intake increases the concentration of riboflavin in the liver (Griffin and Baumann, '46), as well as that of most other B vitamins (Wright and Skeggs, '46), and the present data indicate that the retention of vitamin A is similarly improved when the intake of food is restricted. Another point of similarity between vitamin A and the B vitamins lies in the adverse effect on vitamin storage of proteins deficient in certain essential amino acids. Diets low in tryptophane impair the storage of riboflavin and pyridoxine [6] and the present experiments indicate in a preliminary way that this may also be true of vitamin A. Rats on

[6] Sauberlich and Griffin, unpublished results.

the diet low in tryptophane retained less vitamin A than those of comparable size fed a good protein (casein in the low A diet) but an insufficient amount of food (table 1, series A). After 30 days of depletion, the low calorie rats retained 196 μg of an original 229 μg of vitamin A, as compared to 146 μg on the diet low in tryptophane. The losses of vitamin A were therefore 33 and 83 μg of the vitamin on the 2 diets, respectively.

The present experiments furnish some basis for evaluating a previous conclusion that thyroid enhances the conversion of carotene into vitamin A (Johnson and Baumann, '47b). Normal rats fed 40 μg of carotene daily for 2 weeks stored an average of 52.3 μg of vitamin A in the livers and kidneys in contrast to an average storage of 92.5 μg by hyperthyroid rats receiving similar amounts of carotene. The 2 groups of rats were not significantly different in size. Since it is now clear that the need for vitamin A increases somewhat when the basal rate goes up, it follows that the true effect of the thyroid principle upon the conversion of carotene to vitamin A must have been even greater than that indicated by the differences in vitamin storage observed.

SUMMARY

1. Rats whose growth was restricted by diets inadequate in calories, thiamine or tryptophane retained more hepatic vitamin A than control rats that grew normally.

2. Comparisons between rats of similar size suggested that metabolic rate also influenced vitamin A depletion; desiccated thyroid hastened the depletion of vitamin A reserves somewhat, while thiourea and thiouracil delayed it. Quantitatively, the effect of a 3-fold increase in metabolic rate was less important for vitamin A retention than a decrease in the growth rate by 50%.

3. In normally growing rats a decrease in the hepatic reserves of vitamin A was accompanied by an increase in the amount and concentration of the vitamin in the kidney. No such increase occurred in the kidneys of rats whose growth

was restricted during the depletion period. The concentration of vitamin A in the kidney was particularly low in rats exposed to thiourea.

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IRON AND COPPER METABOLISM OF YOUNG WOMEN ON SELF-SELECTED DIETS[1]

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Few copper metabolism studies have been made with adults on self-selected diets. Two of these have been reported by Leverton ('39) and Leverton and Binkley ('44). In the earlier study 24 1-week metabolism studies which involved the weighing and analysis of all the excreta were made of young women between the ages of 16 and 25 years on self-chosen diets. Results indicated that there was a relation between the level of copper intake and the amount of copper stored. Whenever the intake was less than 2 mg there was a negative balance. From this study it was concluded that a maintenance allowance of 2 mg of copper should be allowed girls between the ages of 16 and 25 years; if more is present in the diet, it will be stored.

In the later study copper metabolism experiments covering 95 1-week periods were made on 65 young women on self-chosen diets. Also, a long-term study was made on 4 young women on an adequate constant diet. The results were similar to those of the first study, i.e., as the copper intake increased, a large proportion of each increase was retained. It was also concluded that a daily allowance of 2.0 to 2.5 mg of copper is adequate and that this amount can be obtained from diets of medium nutritive value.

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[2] In partial fulfillment of the Master of Science degree in Food and Nutrition. At present located at Stephen F. Austin State Teachers College.

A daily iron requirement of 12 mg for young adults is an accepted recommendation.

Because of the frequent occurrence of nutritional anemia in this age group, it seemed desirable to determine both the copper and the iron intake and output on self-selected diets.

PROCEDURE

The subjects used in this study were young college women between the ages of 17 and 22 years who were living in the Home Management House Duplex, North Texas State College, at the time of their participation in the study. All food, feces and urine were analyzed. A serving of each food from each meal, similar in all respects to that eaten by the college women, was collected at the same time that the subjects were served at the table. One-fourth of a glass of milk was collected daily in a similar manner, thus giving 1 homogeneous milk sample for analysis. A record was kept of the number of glasses of milk consumed by each girl each day since this was the only variable in the daily food intake. Suitable additions were then made to each individual's food intake to include the milk. After weighing the food to get the total weight, the food was macerated in the Waring Blendor, and a known fraction of the weight was removed into 1 common jar and kept in the refrigerator until analysis could be made. In some instances meals were eaten outside the house. In this case duplicates of the foods eaten were collected and analyzed separately. Appropriate corrections were made for that individual's food intake for the day.

Carmine was given to mark the feces. After weighing, the feces were macerated in the Waring Blendor and refrigerated. The urine was collected directly in 4-liter amber bottles containing a preservative.

For the calibration curve a standard iron solution was prepared by dissolving 1 gm of electrolytic iron in 1 liter of 10% sulfuric acid (1 mg of iron per milliliter). The Cenco-Sheard-Sanford photelometer was set at 100 with a blank

solution and readings were taken on the standard solutions, using a 515 mμ filter.

The calibration curve for copper was prepared in the same manner, using a standard copper solution in the place of the standard iron solution. The standard copper solution was made by weighing out 3.93 gm of copper sulfate crystals ($CuSO_4 \cdot 5\ H_2O$), dissolving in water and diluting to 1 liter (1 mg of copper per milliliter). The readings were also made on a Cenco-Sheard-Sanford photelometer, using a 515 mμ filter.

Recoveries ranged from 93 to 106% for iron and 92 to 108% for copper.

From the macerated food of known weight, a 15-gm sample, plus allowance for the 1% metaphosphoric acid, was taken for analysis. The sample was weighed into a large porcelain crucible, 10 ml of 50% ammonium acetate solution was added, and it was evaporated to dryness and ashed in the electric muffle at a temperature not exceeding 500°C. (Stone, '42).

Simultaneous determinations of iron and copper were made, using Parker and Griffin's ('39) method of combining the dipyridyl for the estimation of available iron with McFarlane's ('32) carbamate method for the determination of copper in organic materials.

Liter samples of tap water were ashed and also analyzed for iron and copper. There was no detectable iron or copper in 1 liter of tap water. Consequently, the amount of water consumed by these subjects was not important from the standpoint of iron and copper intakes.

DISCUSSION

Because of the close nutritional relationship between iron and copper, both elements were determined in the food, feces and urine of 17 young college women. The girls in each group had the same self-chosen diet with a few exceptions. For instance, M.J.C., L.D.W. and M.G.M. (study I-A) were in the first 5-day study. M.J.C. and L.D.W. had the same self-chosen diet as M.G.M., with the exception of 2 lunches which

they had away from the house. While these 2 lunches did not give the same iron and copper values as the lunches served in the house, they did not materially affect the average daily intake.

Due to unavoidable conditions, only one subject (L.H., study I-B) was on the diet for the whole 5-day period. The self-selected diets varied greatly in the daily iron and copper contents; in fact, so much so, that the 2 girls, P.A. and M.J., who participated on the first 3 days of the period, had much lower daily average intakes than G.R., who was on the diet the last 3 days. The fact that L.H. was on the diet for the full 5 days also gave her lower iron and copper daily average intakes than G.R.

The other variation in the intakes of iron and copper was caused by the different amounts of milk consumed by each of the subjects. Both the first and second studies, covering 5 and 4 days, respectively, included the variable milk consumption of the individual girls. The daily average milk intake was from 0 to 3½ cups. This milk contributed from 0.0 to 1.7 mg of iron and from 0.0 to 2.9 mg of copper to the total daily intakes of these 2 minerals.

The lowest iron intake was observed in study II-A, followed by study I-A and study II-B, with the largest iron intake in study I-B, table 1.

The copper intake did not follow the same sequence of increase which occurred with the iron. In general, the lowest intake of copper was found in the study I-A group, with both groups of study II showing slightly larger amounts consumed. L.H. and G.R. from study I-B, and E.R. of study II-B had the highest average daily intakes of copper. The average daily intakes of iron and copper in food and milk were 6.6 to 13.6 mg with an average of 9.6, and 6.5 to 13.0 with an average of 8.1 mg, respectively.

Leverton and Binkley ('44) found that average daily intakes of 10.4 and 2.7 mg for iron and copper, respectively, on self-selected diets maintained a positive balance for their subjects. The daily average intakes in the present study

TABLE 1

Iron and copper content of food, urine and feces with calculated retention.

NAME	DAYS	INTAKE		EXCRETION						RETENTION			
		Food plus milk		Urine		Feces		Total		Iron		Copper	
		Total		Total		Total		Total					
		Iron	Copper	Iron	Copper	Iron	Copper	Iron	Copper	Total	Average	Total	Average
		mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
						Study I-A							
M.G.M.	5	44.8	40.5	0.2	0.1	5.9	3.7	6.1	3.8	38.7	7.7	36.7	7.3
M.J.C.	5	37.7	32.6	1.7	0.4	7.0	2.9	8.7	3.3	29.0	5.8	29.3	5.9
L.D.W.	5	41.7	36.3	1.7	0.9	9.5	5.7	11.2	6.6	30.5	6.1	29.7	5.9
						Study I-B							
P.A.	3	35.3	24.2	0.4	0.3	3.5	2.0	3.9	2.3	31.4	10.5	21.9	7.3
G.R.	3	40.8	38.9	0.3	0.4	3.7	1.9	4.0	2.3	36.8	12.3	36.6	12.2
L.H.	5	60.4	49.8	0.3	0.4	6.4	1.7	6.7	2.1	53.7	10.7	47.7	9.5
M.J.	3	35.3	24.2	0.6	1.1	5.5	4.0	6.1	5.1	29.2	9.7	19.1	6.4
						Study II-A							
M.H.B.	4	26.6	30.6	1.6	1.3	3.5	0.9	5.1	2.1	21.5	5.4	28.5	7.1
F.D.	4	26.4	30.4	2.0	1.6	2.9	3.1	4.9	4.7	21.5	5.4	25.7	6.4
M.R.	4	27.9	32.1	1.3	0.3	5.1	3.3	6.4	3.6	21.5	5.4	28.5	7.1
C.R.	4	29.1	33.1	2.0	0.4	4.9	3.3	6.9	3.7	22.2	5.6	29.4	7.4
M.C.	4	28.9	32.9	0.9	1.1	6.9	3.8	7.8	4.9	21.1	5.2	28.0	7.0
						Study II-B							
D.B.	4	42.0	33.8	1.2	0.2	3.5	1.6	4.7	1.8	37.3	9.3	32.0	8.0
E.R.	4	46.2	40.7	2.1	2.7	10.2	4.6	12.3	7.3	33.9	8.5	33.4	8.4
L.B.	4	44.1	37.2	0.8	0.1	4.0	1.7	4.8	1.8	39.3	9.8	35.4	8.8
B.G.	4	43.6	36.4	1.3	1.2	6.1	2.6	7.4	3.9	36.2	9.1	32.5	8.1
B.K.	4	42.0	33.8	3.2[1]	1.5	7.9	3.6	11.1	5.1	30.9	7.7	28.7	7.2

[1] Represents 3 days, as 1 day's collection of urine was lost.

differed from these values by 0.8 mg for iron, and by 5.4 mg for copper. The copper of the diets in the present study is much higher than Leverton and Binkley's ('44). This is apparently a regional effect upon the composition of food since this was also found to be true of Nursery School lunches analyzed.

In general, all of the subjects showed an increased retention of both iron and copper when these elements were present in greater amounts in the diet (table 1) although these increases were not consistent. This is in accord with the findings of Leverton and Binkley ('44), that as the intakes of iron and copper increased in the diet, the retention of both elements increased. The total urinary iron ranged from 0.2 to 3.2 mg, and the fecal iron from 2.9 to 10.2 mg. However, the excretion of iron was higher than that of copper in both the urine and feces with a wider range for copper than for iron. These figures indicate that the total excretion of iron was greater than that of copper in urine and in feces.

SUMMARY

The daily intakes of iron and copper in food and milk were 6.6 to 13.6 mg, with an average of 9.6, and 6.5 to 13.0 with an average of 8.1 mg, respectively.

There were no consistent increases in the excretion of iron and copper when the intakes increased. In all instances the total excretion of iron exceeded that of copper. However, increased intakes of both iron and copper usually resulted in increased retentions of each.

All of the 17 young college women on self-selected diets were in positive iron and copper balance.

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SUITABILITY OF THE BOURQUIN-SHERMAN DIET FOR RIBOFLAVIN ASSAYS AND RESULTS OBTAINED IN THE ASSAY OF EVAPORATED MILK

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TWO FIGURES

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The Bourquin-Sherman technic and various modifications thereof are commonly employed in biological assays for riboflavin. In recent years the use of the riboflavin deficient basal diet developed by Bourquin and Sherman ('31) has been questioned. Gyorgy et al. ('34), Street ('41), Lindholm ('38) and El Sadr et al. ('40) have presented evidence of deficiencies in this basal diet of B factors other than riboflavin. As a result of the observations of these workers, other materials have been proposed by them and by Wagner et al. ('40) to supplement or replace the alcoholic extract of wheat used by Bourquin and Sherman to supply the members of the B-complex exclusive of riboflavin.

It is possible that all of the basal diets designed for riboflavin assay fall short of the ideal in that they may not (a) be entirely free from riboflavin, or (b) contain all other nutrients necessary for the rat. While claims of superiority of other basal rations over the Bourquin-Sherman diet are not without support, Day and Darby ('47) have recently published the following statement concerning the latter: "For assays wherein the growth is somewhat limited, and such should be the case in a quantitative riboflavin assay by the rat growth method until the other factors of the diet have been more

carefully studied, growth elicited by a supplement to the Bourquin-Sherman diet may be interpreted as a measure of riboflavin.''

The purpose of this investigation was twofold, namely, (a) to investigate further the suitability of the Bourquin-Sherman basal diet for the determination of the riboflavin value of foods, and (b) to obtain a measure of the riboflavin value of evaporated milk based upon the use of this basal diet. Although there have been a number of reports on riboflavin values for fluid whole milk determined by the rat growth assay method, figures for only a few such assays are available for evaporated milk (Todhunter, '32; Henry et al., '40).

EXPERIMENTAL PROCEDURE

Forty-eight young albino rats, 21 to 22 days of age, equally divided as to sex, and weighing 40 to 50 gm, were placed on the Bourquin-Sherman basal ration. Thirty-six of these were carried to depletion and 12 were supplemented immediately. The latter were divided into 2 groups, each group consisting of 3 males and 3 females. One group received pure riboflavin in addition to the basal diet, and the other group pure riboflavin plus thiamine, pyridoxine, calcium pantothenate and choline. The riboflavin was administered in a 20% alcoholic solution 3 times weekly in amounts equivalent to 75 μg daily. The other vitamins, with the exception of choline, were mixed and likewise administered in a 20% alcoholic solution 3 times weekly. The daily equivalents for these vitamins were as follows: thiamine and pyridoxine, 30 μg each, and calcium pantothenate, 150 μg. The choline was incorporated in the diet in the amount of 1 gm per 1000 gm of the diet, a procedure recommended by Miller ('43).

The 36 unsupplemented rats were considered depleted when they reached a level where they gained less than 6 gm in 2 weeks. This required from 32 to 34 days. The rats were then divided into 6 groups, each group consisting of 3 males and 3 females. Three of the groups were supplemented with pure riboflavin in a 20% alcoholic solution fed 3 times weekly in

amounts equivalent to 3, 6 and 9 μg daily. A fresh supply of the riboflavin made up according to the method of Wagner et al. ('40) was prepared each week and stored in a dark bottle in the refrigerator. The other 3 groups were supplemented with undiluted evaporated milk in amounts providing presumptive levels approximating those of the pure riboflavin. These estimated quantities were supplied by feeding 3 times weekly a daily equivalent of 0.8, 1.6 and 2.4 ml.

RESULTS AND DISCUSSION

The average growth rates of the 2 groups of rats on the Bourquin-Sherman diet supplemented from the beginning are reported as graphs in figure 1. The average gain for a 9-week

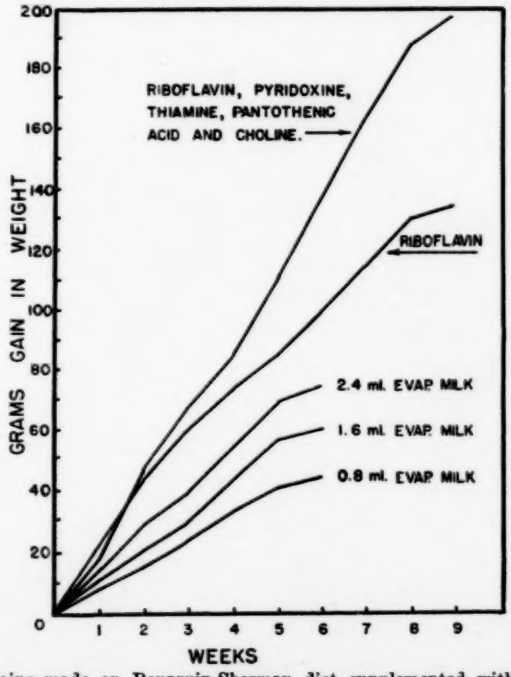

Fig. 1 Gains made on Bourquin-Sherman diet supplemented with evaporated milk; with riboflavin alone; and with riboflavin plus pyridoxine, thiamine, pantothenic acid and choline.

period of the group supplemented with riboflavin alone was 133.7 gm, while the group receiving riboflavin, thiamine, pyridoxine, calcium pantothenate and choline made an average gain of 198.8 gm. The data would seem to indicate that the Bourquin-Sherman diet has B vitamin inadequacies in addition to riboflavin. Further evidence of a deficiency of some

TABLE 1

Growth response of riboflavin-deficient rats to graded levels of pure riboflavin and evaporated milk as a source of riboflavin.

DAILY LEVEL OF SUPPLEMENT	NUMBER OF RATS	AVERAGE GAIN PER WEEK (GM)						AVERAGE GAIN	
		1	2	3	4	5	6	For 6-week period	Daily for 6 weeks
µg									
		Pure riboflavin as the supplement							
3	6	8.4	6.5	3.8	6.6	3.8	2.1	31.2	0.74
6	4[1]	10.8	10.2	8.1	11.0	10.6	3.0	53.7	1.28
9	6	12.3	10.8	11.1	13.9	10.8	5.9	64.8	1.52
ml		Evaporated milk as source of riboflavin							
0.8	6	8.1	7.6	7.6	10.3	8.0	3.2	44.8	1.06
1.6	4[2]	11.7	8.7	7.0	16.0	12.2	3.2	58.8	1.39
2.4	4[3]	15.3	13.3	10.2	16.9	12.0	5.9	73.6	1.75

[1] A male rat in this group died during the fourth week of the assay period.

[2] A male animal in this group lost weight for a period of 2 weeks, due to an unexplainable illness.

[3] A female in this group, presumably due to animal variation, made an abnormally high weight gain.

The growth rates of the above rats were not included when calculating average gains in weight. To insure an equal number of males and females in each group 1 rat of the opposite sex was eliminated in each case.

nutrient other than riboflavin was the occurrence of slight alopecia in 2 of the rats supplemented with riboflavin alone. Alopecia was also observed in 2 of the rats subjected to riboflavin depletion. One of these rats later received riboflavin supplementation at the 6 µg level, and the other evaporated milk at the 1.6 ml level. The alopecia disappeared in about 2 weeks in the rat receiving evaporated milk, but the one on

the riboflavin supplement remained unchanged throughout the assay period. At the end of the assay period the amount of riboflavin was increased to 200 μg daily with no effect. There was then administered in addition to the riboflavin 90 μg of pyridoxine, with a resulting growth of hair. It has been suggested by Bessey and Wohlbach ('39) and by Clark et al. ('40) that one of the deficiencies of the Bourquin-Sherman diet is pyridoxine, and the observations just recorded give support to this suggestion.

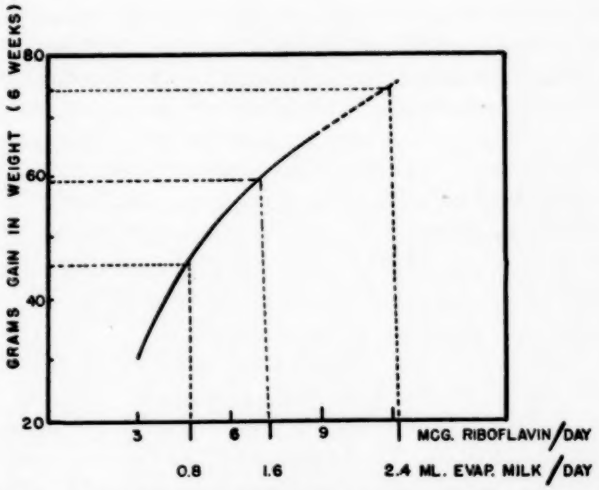

Fig. 2 Curve of response constructed from average gains of rats on graduated doses of pure riboflavin. Dotted lines indicate how curve is used to convert weight gains of rats fed evaporated milk into riboflavin values.

Table 1 shows the growth response of riboflavin depleted rats to graded amounts of pure riboflavin and evaporated milk. The graphic representation of the growth rates on evaporated milk has been included in figure 1. Figure 2 shows the curve of response constructed from the average gains of the riboflavin supplemented rats for a 6-week period. The rate of gain was found to be roughly proportional to the

amounts of supplement fed and there was no overlapping of average weekly gains for the different levels ingested.

In order to interpret the gains on evaporated milk as micrograms of pure riboflavin, they were plotted on the curve of response, and from the points of intersection, perpendicular lines were dropped to the base line and their locations were read directly as micrograms of riboflavin. The riboflavin content of the highest level of milk (2.4 ml) was obtained by extrapolation of the curve, since the growth rate on this level of milk exceeded that of the highest level of pure riboflavin fed. The average of the riboflavin values for the 3 levels of milk was found to be 5.1 μg per milliliter. This is higher than the values for evaporated milk reported by Todhunter ('32) and Henry et al. ('40). However, this study is not wholly comparable with that of the above investigators. Todhunter's work was done before pure riboflavin was available for use as a reference standard, and Henry et al. used a different basal diet. Also the evaporated milk assayed by Henry et al. appears to have been more dilute than the commercial product used in this study. The latter was stated to contain not less than 29.5% total solids.

The riboflavin value obtained for evaporated milk in the present investigation is in close agreement with the upper limits of riboflavin bioassay figures reported for whole fluid milk (Kunerth et al., '37; Lunde et al., '39) when correction is made for the concentration of the evaporated milk. This suggests that riboflavin is not lost in the evaporation processes. In general, the riboflavin values reported for both whole fluid milk and evaporated milk based upon microbiological and fluorometric measurements are lower than those obtained by bioassay (Whitnah et al., '37; Daniel and Norris, '44). This can probably be explained on the basis of deficiencies other than riboflavin in the basal diets used, and the presence in milk of the deficient factors. Evidence has been presented in this study of insufficient amounts of B vitamins other than riboflavin in the Bourquin-Sherman basal diet.

SUMMARY

An investigation was made which included:

1. A study of the adequacy of the Bourquin-Sherman riboflavin deficient diet with respect to certain other members of the vitamin B complex.

2. The use of the Bourquin-Sherman basal diet in the determination of the riboflavin content of evaporated milk.

The growth rate of a group of rats on the Bourquin-Sherman diet supplemented with riboflavin alone was considerably less than that of a similar group of rats receiving, in addition to riboflavin, supplements of thiamine, pyridoxine, calcium pantothenate and choline. The results indicate a deficiency of the basal diet in one or more of the B vitamins in addition to riboflavin. Some evidence was obtained which suggested that pyridoxine was suboptimal.

In measuring the riboflavin content of evaporated milk, pure riboflavin was used as a reference standard. The value obtained was 5.1 μg per milliliter. This value is higher than that obtained for evaporated milk in earlier rat growth assays employing somewhat different technics. It is, however, in close agreement with the upper limits of riboflavin figures for whole fluid milk assayed by the Sherman-Bourquin method, when the concentration of the evaporated milk is taken into account. This suggests that riboflavin is not lost in the evaporation processes.

In general, recent bioassay riboflavin figures for milk have been found to be higher than those obtained by the microbiological and fluorometric methods. A probable explanation, in the light of the results of this and other investigations, is the presence in milk, in addition to riboflavin, of B factors not present in optimal amounts in the basal diet.

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PROCEEDINGS OF THE TWELFTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

HADDON HALL AND MUNICIPAL CONVENTION HALL,
ATLANTIC CITY, NEW JERSEY, MARCH 14–19, 1948

COUNCIL MEETINGS

Council meetings were held at Haddon Hall on Sunday and Monday, March 14 and 15. Formal actions of the Council are reported in the minutes of the business meetings.

SCIENTIFIC SESSIONS

The scientific program consisted of 6 half-day sessions of papers grouped according to topic. A total of 65 papers was presented, and 7 were read by title. In addition, the Tuesday afternoon session was devoted to the Joint Session of the Federation.

BUSINESS SESSIONS

Two business meetings were held, one at 10:00 P.M. Tuesday evening, March 16, following the Institute Dinner; the other at 4:30 P.M. Thursday, March 18, following the scientific program. President R. M. Bethke presided at these meetings.

Tuesday, March 16, 10:00 P.M. The Secretary gave a brief statement on the Institute membership, noting the death of Fred C. Koch on January 26, 1948. The report of the Treasurer was presented by N. R. Ellis. The Auditing Committee (O. L. Kline and H. R. Bird) reported that the Treasurer's books were in order. The Treasurer's report was approved.

Dr. George R. Cowgill, Editor of THE JOURNAL OF NUTRITION, gave a brief report concerning the affairs of the JOURNAL as follows:

Beginning with January, 1947, THE JOURNAL OF NUTRITION has had an allowance of 120 pages per issue instead of the former 100 pages. The editor had asked The Wistar Institute to make this increase in order to permit more prompt publication of the acceptable material submitted. There seemed to be a definite trend in the direction of greater number of papers being offered. The recommendation to The Wistar Institute was based on the records indicating this trend. This increase meant a change in volume size from one of 600 pages to one containing 720 pages. As usual, 2 volumes were published during the year, namely, volumes 33 and 34; they contained 121 articles. During the year 166 papers were submitted for consideration. The average number of papers per issue was 10, the same as for the previous year; the average number of pages per article was the same as that for the previous year, namely, 12.2 pages.

In keeping with the plan to share, whenever possible, any success of the Journal with the membership of the American Institute of Nutrition, the editor has from time to time discussed with Dr. Edmond J. Farris, Executive Director of The Wistar Institute, whether the subscription rate to members of the society might not be reduced. Whether this could be done was essentially a financial matter. Sometime ago it was decided that if our volume of advertising should be sufficient and should prove to be fairly constant and the number of subscriptions should continue to be sufficient, something along this line might be done. For some time now these conditions appear to have been met. As a result, the Editor proposed recently to The Wistar Institute that, beginning with volume 36, the first issue of which appears in July, 1948, the subscription rate for members of the American Institute of Nutrition be set at one-half the regular rate. This request has been granted. This agreement will remain in effect as long as general income (including the advertising revenue) will be sufficient to offset satisfactorily the cost of printing the Journal.

The Editor's report was approved and the members extended to Dr. Cowgill and the Editorial Board a vote of sincere appreciation for their efforts.

Dr. R. E. Johnson described the organization of various Pathological Registries in the Army Institute of Pathology and proposed that the American Institute of Nutrition sponsor a Registry of Nutritional Pathology, since a request by an interested Scientific Society to the Scientific Director of Registries is a necessary step in setting up such a Registry. Dr. Johnson explained the value of this Registry and pointed out that only a token contribution would be needed from the Institute since the Registries are financed in other ways. After some discussion it was decided that the President should appoint a small *ad hoc* committee (preferably composed of a medical nutritionist, an animal nutritionist, a biochemist, and 1 person familiar with Army organization) to consult with the Scientific Director, American Registry of Pathology, concerning the establishment of a Registry of Nutritional Pathology.

The Secretary reported on correspondence with Dr. Leslie J. Harris who has been empowered by the (British) Nutrition Society to carry on negotiations leading to the establishment of an International Union of Nutrition Societies. Dr. Harris suggested that a Provisional Committee (consisting of 1 or more representatives of each of the 13 countries contacted) should meet in England during the summer of 1948 to explore the situation. The membership expressed approval of sending a delegate to this meeting from the American Institute of Nutrition provided some method could be found for meeting the expenses involved.

The President reported that the Nutrition Foundation had expressed a desire to provide an award of One Thousand Dollars each year to a scientist making an outstanding contribution to nutrition and had asked the Institute to administer the award. The following report was made by the Committee appointed to consider this matter:

Report of the Special Committee on Rules and Regulations Governing the Annual Award Made Available by the Nutrition Foundation, Inc.

The Committee makes the following recommendations:

1. That the American Institute of Nutrition accept with sincere appreciation the offer of The Nutrition Foundation, Inc. to provide annually an award of One Thousand Dollars for outstanding accomplishments in the science of nutrition, and the expenses of the recipient, or recipients, incident to attendance at the annual meeting at which the award is made, and the cost of preparation of an appropriate scroll, the cost of travel and the preparation of the scroll to be within the limit of the sum provided for these purposes.
2. That the award be known as the Research Award of the American Institute of Nutrition.
3. That the award be made in recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition.
4. That it be made to an investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in approximately the year preceding the annual meeting of the Institute or, who has published a series of contemporary papers of outstanding significance.
5. That, as a general policy, the award be made to 1 person. If, in the judgment of the Jury of Award an injustice would otherwise be done, it may be divided among 2 or more persons.
6. That normally, the preference be given to research workers in the United States and Canada, but that investigators in other countries are not to be excluded from consideration. (Thus, it might be appropriate to give consideration to an investigator who has done outstanding work and who is a resident in, or who is visiting, the United States or Canada for a period of time.)
7. That there be no limitation as to age.
8. That membership in the American Institute of Nutrition is not a prerequisite of eligibility for the award.
9. That the award be made at the annual meeting of the American Institute of Nutrition.
10. That the recipient be presented with a suitably inscribed scroll signed by the President of the American Institute of Nutrition.

11. That if, in the opinion of the Jury of Award, no suitably qualified candidate is nominated in a given year, it shall so report to the President of the Institute and no award shall be made.

12. That an announcement of the award be made each year in the July, October, November and December issues of The Journal of Nutrition, including a request that nominations be sent to the Chairman of the Nominating Committee before January 15 of the year in which the award is to be made.

13. That a Nominating Committee of 3 members be appointed by the President to receive and to make nominations. To maintain a continuity of experience and to provide for rotation of membership, the first committee shall consist of 1 member appointed for a 3-year term, 1 for a 2-year term and 1 for a 1-year term. Thereafter, members shall be appointed for 3-year terms. The member with 1 year yet to serve shall be chairman.

14. That a Jury of Award of 5 members of the Institute be named by the President, in accordance with the provisions stated below, to select a recipient.

To provide for rotation of membership and at the same time to maintain a continuity of experience, the first Jury shall consist of 2 members appointed for 3-year terms, 2 for 2-year terms, and 1 for a 1-year term. Thereafter all appointments shall be for 3-year terms. After a juror has served a term of any length he shall not be eligible for another appointment until after the lapse of 1 year. The President of the Institute shall be Chairman of the Jury but shall not be entitled to vote.

When a vacancy occurs on the Jury, the President shall fill the vacancy by appointment for the unexpired term.

If a juror is nominated for the award, he shall resign his appointment immediately and the President shall fill the vacancy by appointment for the unexpired term.

15. That the Jury of Award use the following procedure in receiving and considering nominations.

Promptly after January 15 of the year in which the award is to be made, the Chairman of the Nominating Committee shall transmit all documents to the Chairman (President of the Institute) of the Jury of Award. The Chairman of the Jury will then transmit the nominations with supporting data to the members of the Jury requesting prompt review and compliance with the following procedure:

The Chairman shall first ask the members of the Jury whether any candidate is worthy of the award. If a majority of the members answer in the negative, no award will be made. If a majority answer in the affirmative, then the Jury shall at once proceed to ballot, each member of the Jury indicating to the Chairman his first, second, and third choice. If a majority of the members of the Jury select as first choice the same person from the list of nominees, such a choice shall be final and that person shall be designated as the recipient of the award. If there is no such agreement, the Chairman shall weigh each first choice as 3, each second choice as 2, and each third choice as 1, and shall total the score of each nominee as obtained by this method. He shall then prepare a list of preferably not more than 7 of those having the highest total score. Balloting shall be continued on the candidates so selected. On each subsequent ballot each member of the Jury shall vote and the Chairman shall eliminate the candidate having the lowest point score until but 2 remain. From these 2 the Committee shall select the recipient. However, if on any ballot any candidate is the choice of a majority of the members of the Jury, he shall be declared elected.

The final decision of the Jury must be made at least 3 weeks before the Annual Meeting of the Institute.

The President of the Institute shall communicate the decision of the Jury to the recipient.

Respectfully submitted,

WALTER RUSSELL, *Chairman*
GEORGE R. COWGILL
VINCENT DU VIGNEAUD
D. W. WOOLLEY
ARTHUR H. SMITH.

The above report was approved by the members.

The Secretary reported that the Executive Committee of the Federation had taken the following actions:

1. Elected Dr. M. O. Lee as Secretary-Treasurer for the coming year. (Dr. Lee has already established a permanent office in the National Academy of Sciences building in Washington.)

2. Decided that the 1949 meeting should be held in Detroit (week of April 18) and the 1950 meeting in Atlantic City.

Also recommended that the meeting be held every other year in Atlantic City.

3. Recommended that the Joint Session of the Federation be held in the evening.

4. Recommended that the Federation assessment be set at Three Dollars per member, all of this to be used in support of *Federation Proceedings.*

5. Recommended that the Federation By-Laws be altered so as to permit the Control Board to elect a Chairman.

6. Recommended that no reprints of the abstracts published in *Federation Proceedings* be made available to the authors.

Items 3, 4, 5, and 6 were approved by the members of the Institute.

The financial obligations of the Institute members for the year July 1, 1948–June 30, 1949 were fixed at Nine Dollars (Five Dollars for *Journal of Nutrition,* Three Dollars for *Federation Proceedings,* One Dollar for Institute of Nutrition dues).

A motion to enlarge the ballot by including space for the suggestion of 5 names for the Nominating Committee was approved.

The members approved the proposition that the Executive Committee should advise its A.A.A.S. council representative to urge that legislation creating a National Science Foundation should specify that the Director should be selected by the President from a list provided by the National Academy of Science.

The Program Committee (consisting of the Secretary and members appointed by the President) was empowered to construct the program of the annual meeting in such a way as to meet any space limitations imposed on the Federation Meeting and to limit the number of papers presented if this move became essential.

It was decided to hold a Dinner annually and that this function should not be restricted to members if adequate facilities were available.

The President appointed Drs. T. H. Jukes and N. B. Guerrant as Tellers.

The meeting adjourned at 12:00 P.M.

Thursday, March 18, 4:30 P.M. The Tellers reported that the following officers had been elected for 1948–1949:

President, E. M. Nelson
Vice-President, C. G. King
Treasurer, N. R. Ellis
Secretary, J. H. Roe
Councillor, E. N. Todhunter
Associate Editors, R. H. Barnes
P. L. Day
H. H. Mitchell

The following new members were elected on recommendation of the Council:

Chow, B. F.
Dodds, Mary L.
Engel, R. W.
Everson, Gladys
French, C. E.
Frey, C. N.
Heinle, R. W.
Higgins, G. M.
Johnston, Frances Ann
Leichsenring, Jane M.
McCoy, R. H.
Rusoff, L. L.
Shaw, J. H.
Todd, W. R.
Youmans, J. B.

The Council was given authority to designate an official representative to the Provisional Committee meeting this summer to consider an International Union of Nutrition Societies, provided financial arrangements for such a delegate could be completed.

The membership voted that the Secretary should send the following resolution to the President of the United States, President of the Senate, and Speaker of the House:

The American Institute of Nutrition hereby records its strong disapproval of the methods employed by the Committee on Un-American Activities of the House of Representatives in publicly implying, without affording proper opportunity for reply, that Dr. E. U. Condon, Director of the National Bureau of Standards, had failed properly to safeguard secrets vital to the safety of the United States. The Committee on Un-American Activities violated basic civil rights in thus making implied accusations not proved by

facts in evidence. The Institute wishes to emphasize that the fabric of cooperation between scientists and their government, so carefully and strongly woven during the war and so important for the welfare and safety of the United States will be seriously weakened if the loyalty and integrity of citizens can be publicly questioned without furnishing acceptable substantiating evidence in conformity with established judicial procedures.

Dr. E. M. Nelson of the Joint Committee on Nomenclature reported that the Committee proposed using the term "folacin" as synonymous with pteroylglutamic acid. The Institute approved the request of the Committee that publicity be given this suggestion before a final decision was reached.

It was announced that the first International Congress on Biochemistry would be held in London in August, 1949.

The Institute gave a hearty vote of thanks to Drs. Lee and Chambers for their fine efforts in organizing and arranging the meeting.

President Bethke appointed the following Nominating Committee for 1948–1949:

H. G. Day, Chairman
H. Hunscher
H. J. Deuel
E. W. Crampton
F. J. Stare

The meeting adjourned at 5:30 P.M.

DINNER AND PRESENTATION OF AWARDS

The Institute held its annual dinner on Tuesday evening, March 16, at the Jefferson Hotel. Dr. Frank G. Boudreau gave a very interesting and stimulating talk on the opportunities for improvement of world conditions through better nutrition.

The Mead Johnson and Company Prize was presented to Dr. Fritz Lipmann and the Borden Award to Dr. Charles A. Cary.

Respectfully submitted,
H. E. CARTER, *Secretary*
American Institute of Nutrition

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